

RESEARCH ARTICLE

Longitudinal Characterization of Giant ANK2-Depleted Monkeys Suggests Neurodevelopmental-Disorder-Like Phenotypes

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The ANK2 gene mutations are marked risk factors for autism spectrum disorder, one of the neurodevelopmental disorders (NDDs) that often extends into adulthood and has a complex etiology involving genetic and environmental factors. ANK2 encodes 2 major isoforms, AnkB-220 (220-kDa isoform of ankyrin-B) and giant AnkB-440. We previously generated a targeted knockout (KO) of giant AnkB-440 in 2 cynomolgus and 2 rhesus monkeys. While no autism-spectrum-disorder-like phenotypes were observed during infancy, we found marked brain volume loss. In this study, we conducted a longitudinal multimodal study in these giant ANK2 KO monkeys during adolescent and young adulthood. Behavioral results from these giant ANK2 KO monkeys revealed increased locomotor activity, deficient cognition (including working memory, cognitive flexibility, and operant lever-press learning), and impaired emotional regulation and social interaction. Furthermore, the giant ANK2 KO monkeys exhibited persistent structural and functional brain abnormalities, up-regulation of brain triglyceride and glycerophospholipids, and peripheral blood transcriptome signatures of immune dysregulation, which may be related to their behavioral alternations. These observations suggest that giant ANK2 depletion in non-human primates phenocopies aspects of behavioral, neural, and molecular features characteristic of NDDs. This study provides an in-depth exploratory longitudinal characterization of giant ANK2 functions in primate brains, which may contribute to translational research on NDDs.

Introduction

Human neurodevelopment is a prolonged process extending into adulthood. Abnormalities during this period can lead to neurodevelopmental disorders (NDDs), conditions that originate in

development and result in deficits in personal and social functioning [1–3]. Common NDDs include autism spectrum disorder (ASD), intellectual disability (ID), attention-deficit/hyperactivity disorder, and motor disorders. Schizophrenia and neurological malformations are also proposed to belong to this

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spectrum [1–3]. The etiology of NDDs is complex, involving genetic and environmental factors [4–6].

The *ANK2* gene is crucial for neural development, playing a role in axonal guidance, neural circuit assembly [7–12], and stability of inhibitory synapses [13]. It encodes AnkB (ankyrin-B), which has 2 major isoforms: The widely expressed 220-kDa protein (AnkB-220) is found to function in dendritic spines, and the neuron-specific 440-kDa protein (giant AnkB-440) predominates in unmyelinated axons [12,14]. AnkB mutations are linked to peripheral pathologies, such as cardiac dysfunction, epilepsy, and metabolic syndrome [15–17]. Importantly, *de novo* *ANK2* mutations are marked risk factors for ASD [18–21] and are enriched in NDD populations presenting with developmental delay and ID [22–24], supporting *ANK2*'s associations with broader NDDs [22]. The giant *ANK2* isoform (AnkB-440) encompasses the full AnkB-220 sequence along with additional exons, suggesting that mutations in AnkB-220 could also impact the giant *ANK2* isoform. To date, however, researches have focused almost exclusively on mutations within the AnkB-220 sequence, leaving the functions of giant *ANK2* in the nervous system largely unexplored.

Previous studies using rodents revealed AnkB's role in spine regulation, neuritogenesis, and axonal transport and development [10,12,14]. *ANK2* knockout (KO) mice showed disrupted neurogenesis and neuronal migration, axon tract loss, enlarged lateral ventricles [7,25,26]. Mice with a mutation or deficiency in giant AnkB-440 exhibited ASD-like behaviors and aberrant structural connectivity probably due to the gain of axon branching [10]. However, translating findings from mice to humans is challenging because of significant interspecies differences in genetics and neurobiology [27]. For example, *SHANK3* or *PINK1* mutant non-human primates (NHPs) exhibited neuronal development disruptions not seen in mice [28,29], highlighting the need for NHPs in neurological research, which share the closest phylogenetic relationship to humans and display similar brain developmental trajectories and architectural features [30–33]. Furthermore, the symptoms of NDDs typically emerge and reach full manifestation during late childhood or adolescence, a developmental period that is markedly compressed in rodents yet significantly extended in primates. Consequently, longitudinal studies in NHPs are indispensable for accurately delineating the complete disease course.

Previously, we generated a targeted KO of giant *ANK2* (AnkB-440) in rhesus monkeys and cynomolgus monkeys [11] to better model human ASD or other NDDs. Surprisingly, we observed pronounced brain volume loss in these KO monkeys. However, behavioral assessments in infancy revealed no significant differences in social interaction, exploratory or stereotypical behaviors, sleep, or activity levels between KO and control monkeys. These phenotypes were dramatically different from those of mice [10]. Although early brain structural abnormalities are clearly evident, the questions of particular relevance in NHPs remain unknown: whether these deficits persist or progressively worsen over time and how giant *ANK2* depletion affects the brain during adolescence and young adulthood.

Guided by our infantile observations, we speculated that giant *ANK2* KO would drive a suite of behavioral, neural, and molecular alterations that unfold across adolescence and into adulthood. To address this, we performed a longitudinal multimodal characterization of previously generated giant AnkB-440 KO monkeys, tracking them from adolescence through young adulthood (Fig. S1). Our analyses revealed heightened activity

levels without stereotypical behaviors, multiple cognitive deficits including impairments in working memory, cognitive flexibility, and operant lever-press learning, as well as persistent structural and functional brain abnormalities. Notably, these mutant animals exhibited pronounced social interaction deficits, emotional dysregulation, and abnormalities in both metabolomic and blood transcriptomic profiles.

Collectively, these findings substantiate the involvement of giant *ANK2* in critical neurofunctional domains and lay a potential platform for unraveling the cross-species mechanisms of giant *ANK2*-related neurological disorders, as well as developing possible therapeutic interventions in primates.

Results

During a 7-year follow-up study, we performed a battery of behavioral tests to assess general symptoms, including neurological function, sleep patterns, exploratory behaviors, and manual dexterity, on the KO monkeys and age-matched wild-type (WT) monkeys (Fig. S1). Partly consistent with our previous observations for these monkeys from infancy to 2 years old [11], we found no significant differences between the 2 monkey groups at adolescent and young adulthood in these paradigms (Fig. S2A to E).

The giant *ANK2* KO monkeys displayed distinct posture and hyperactivity

We adopted an artificial-intelligence-based 3-dimensional (3D) motion recognition framework [34,35] to estimate changes in free-moving behaviors and posture, which might decode the monkey's behavioral sequence with higher granularity in both temporal (1/30 s, 30 fps) and spatial (millimeter-scale) levels (Fig. 1A). Under this framework, videos captured from a precalibrated multiview sampling system were conducted to automatically label and track 3D coordinates of 21 key body parts by a well-trained deep learning model for rhesus monkeys (Fig. 1A) [35]. The model precisely recognized the body parts and reconstructed the 3D skeletons matching the original postures, which ensured the reliability of the system and data for downstream analysis (Fig. 1A) and was used only for rhesus monkeys in this study.

In the subsequent investigation, we observed that the 2 KO rhesus monkeys could be clearly separated from 5 WT rhesus monkeys in the 2D space in the scores plot of principal components analysis (PCA) on the basis of extracted kinematic parameters (Fig. 1B). Group-by-group comparison illustrated that 43 of 46 parameters were significantly increased in the KO rhesus monkeys, including the moving speed and moving intensity of most of labeled body parts, as well as the moving distance per frame and body height (Fig. 1C and D and Table S1; e.g., moving speed of right knee, corrected $P = 0.068$; moving speed and intensity of other parts, corrected $P < 0.05$; distance, corrected $P = 0.014$; height, corrected $P = 0.027$). However, KO rhesus monkeys exhibited similar body length and body angle to those of the WT monkeys (Fig. 1D). We calculated the fold change of the KO rhesus monkeys as the ratio of KO to WT. Nearly half of the kinematic parameters that differed significantly were 2-fold higher in KO monkeys than in WT monkeys (e.g., movement speeds of head and shoulder regions), while the remaining parameters were approximately 1.5-fold higher (Fig. 1E). These data indicated that the KO rhesus monkeys exhibited stronger motional amplitude.

According to the position information in 3D coordinates, the location preference was determined. The location graph

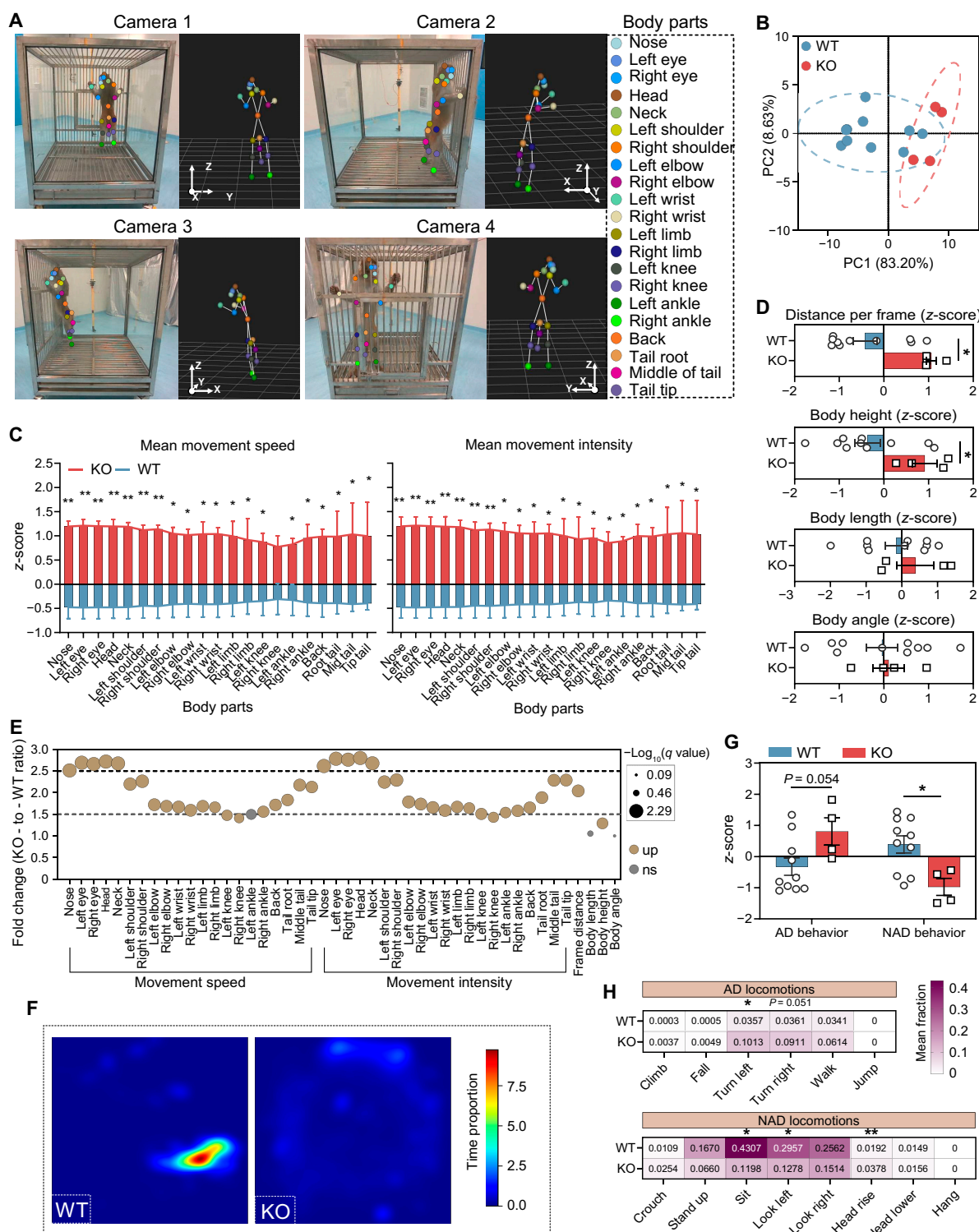


Fig. 1. Immoderate activity and aberrant free-moving behaviors in the giant *ANK2* knockout (KO) rhesus monkeys. (A) Schematic diagram of animal recording using 4 synchronous cameras and the reconstructed skeletons (shown right of the camera pictures) using a well-trained model. The model can precisely recognize 21 body parts (dashed box). (B) Principal components analysis (PCA) score plot for monkeys based on 46 kinematic parameters. (C) Bar plot comparing the mean movement speed and movement energy of each labeled body part showed increased activity in KO monkeys compared to wild-type (WT) monkeys. (D) Bar plot comparing the mean movement distance per frame, mean body height, mean body length, and mean body angle between KO and WT groups. (E) Fold change calculated by the mean of KO to WT ratio for each kinematic parameter. Brown dots denoted significant up-regulation (up), while gray dots denoted no significant (ns) difference. Size of the dot denoted $-\log_{10}(q \text{ value})$. $q \text{ value} = P \text{ value corrected by false discovery rate (FDR)}$. (F) Representative location heatmap of WT and KO monkeys. Color bar presenting the proportion of time spent. (G) Bar plot comparing the apparent displacement (AD) and non-AD (NAD) behaviors showed different body postures and general behavior spectrum between KO and WT groups. (H) Heatmap displaying the mean proportion of AD and NAD behavior. Color bars and figures in the cell presented the mean proportions of each group. $P < 0.05$ and $**P < 0.01$, KO versus WT; multiple t tests with FDR correction for (C) and (D); Student's t tests or Mann-Whitney U test for (G) and (H). Data were presented as means $\pm$ SEM. Rhesus KO group, $n = 2$; rhesus WT group, $n = 5$.

mapping the time proportion spent in any location by the monkeys demonstrated that the KO rhesus monkeys had no obvious position preference. In contrast, the WT rhesus monkey spent more time on a specific point, although each individual was heterogeneous, shown as the location heatmap (Fig. 1F). We analyzed 14 locomotor behaviors and categorized them into apparent displacement (AD) behaviors and non-AD (NAD) behaviors. The AD behaviors included climbing, falling, turning left/right, walking, and jumping, while the NAD behaviors included crouching, standing up, sitting, looking left/right, head rising/lowering, and hanging (Table S2). The KO rhesus monkeys displayed higher proportions in AD behaviors and lower proportions in NAD behaviors (Fig. 1G, AD: $P = 0.054$ and NAD: $P = 0.014$). Specifically, a higher movement fraction on turning left ($P = 0.036$) and turning right ($P = 0.051$) and a lower movement fraction on sitting ($P = 0.020$) and looking left ($P = 0.036$) were detected in KO rhesus monkeys (Fig. 1H). However, it exhibited significantly more head rising than the WT monkeys (Fig. 1H, $P = 0.002$). This behavior characteristic paralleled with the location pattern. We did not find significantly higher stereotypical behaviors in KO monkeys, resembling the infantile observations [11].

Collectively, these results indicated that KO rhesus monkeys exhibited increased activity, altered body postures, and a broad spectrum of general behavioral changes in the same novel environment.

Cognitive function was impaired in the giant ANK2 KO monkeys

As species-specific analysis showed no differences between rhesus and cynomolgus monkeys in performance on the cognitive tasks (Fig. S3), we combined data from both species to maximize the sample size within each experimental group. In the spatial delayed response (SDR) task (Fig. 2A), 4 KO monkeys (from both species) exhibited impaired working memory. In detail, they failed to reach the criterion of 86.7% correct responses when the delay B value exceeded 0 s. The KO monkeys presented a significantly lower percentage of correct responses at $B = 2$ s (Fig. 2B, $P = 0.006$) and also showed a significantly lower maximum B value than the 9 WT monkeys (Fig. 2C, $P = 0.023$). We also compared the average accuracy across all trials with $B > 0$ s using linear mixed-effects model (LMM). The significant fixed effect of delay ($P < 0.001$) revealed that accuracy decreased as the delay increased in both groups (Fig. 2D). In addition, a fixed effect of group was observed, indicating that the KO group performed worse than the WT group across all 5 delays: delay A (0 s, $P = 0.070$), delay B ($P = 0.003$), delay C ($P < 0.001$), delay D ($P < 0.001$), and delay E ($P < 0.001$) (Fig. 2D).

In the visual discrimination and reversal learning (VD/RL) task (Fig. 2E), the 4 KO monkeys presented normal association learning but insufficient cognitive flexibility. LMM revealed no differences in task stage $\times$ genotype interactions for trials to criteria (Fig. 2F) and errors to criterion (Fig. 2G), indicating that the pattern of task-related changes in trials or errors to criterion was comparable across groups. There were significant fixed effects of task stage (trials: $P = 0.027$ and errors: $P < 0.001$) and genotype (trials: $P = 0.040$ and errors: $P = 0.023$) on trials and errors before achieving criteria (Fig. 2F and G). Although post hoc comparisons by Fisher's least significant difference (LSD) test showed no differences in the number of trials or

errors during the VD stage, the KO group performed markedly more trials (Fig. 2F, $P = 0.009$) and errors (Fig. 2G, $P = 0.003$) to criteria than the WT monkeys when the stimulus-reward association was reversed in the RL stage. Likewise, KO monkeys had similar mean error rate relative to WT monkeys in the VD stage, whereas the mean error rate of KO monkeys was significantly higher than that of the WT monkeys in the RL stage (Fig. 2H, $P = 0.026$). A trial-by-trial analysis demonstrated that KO monkeys made 2 times more perseverative errors to achieve the criteria after reversal (Fig. 2I, $P < 0.001$). In addition, the 4 KO monkeys showed significantly lower performance on the operant learning (OL) task compared to eleven WT monkeys, as reflected by the need for more experimenter-guided/manual training trials to reach criterion in KO monkeys (Fig. 2J, $P = 0.044$). This result suggested that the KO monkeys had impaired ability for OL. Taken together, KO of giant *ANK2* in both species (rhesus and cynomolgus) might contribute the underlying cause of cognitive deficits in adolescent monkeys.

Abnormalities of emotional regulation and social interaction in giant ANK2 KO monkeys

To test whether the giant *ANK2* KO would impact the emotion and social interaction of monkeys, we performed emotional response (ER) tests and social interaction tests (SITs) on the monkeys. The mean retrieval latency across 5 sessions showed that the WT group displayed a significantly increased latency when neutral objects were shifted to the fear-inducing rubber cobra ($P = 0.001$), whereas no significant difference was found in the KO group, albeit there was a tendency for increased latency ($P = 0.125$) (Fig. 3A). Consistently, the approach-defense index calculated from behavioral scores also showed a significantly increased defensive behavior of WT monkeys ($P = 0.001$) but not of KO monkeys ($P = 0.125$) (Fig. 3B). These findings indicated that the KO group exhibited altered emotional responses to fearful stimuli and failed to inhibit their food retrieval responses. Note that the KO group had a small number of animals and the values should be interpreted with caution.

We next assessed the latency across the 5 sessions. A group-by-group comparison exhibited that the KO and WT groups had similar food-retrieval latencies in each session regardless of whether it was a neutral or negative condition (Fig. 3C). However, a within-group analysis revealed that the WT monkeys would adapt to the fearful objects, evidenced by a significant reduction of food-retrieval latency in the presence of the 3 stimuli as the testing sessions progressed (Fig. 3C, neutral: $P < 0.001$, spider: $P = 0.011$, and snake: $P = 0.002$). On the other hand, KO monkeys did not exhibit such an adaptation (Fig. 3C). Moreover, the 2 groups showed no difference in latency of neutral trials immediately before and after the snake emerged (Fig. S2E), indicating that the designed order in which negative stimuli were presented was not a factor affecting the results.

The observation from playback of behavioral records under the group-rearing condition showed that the KO monkeys presented a solitary trait (Fig. S2F) and the monkey T105 preferred to stay in the inner room rather than the outside room in the single colony unit (Fig. S2G). Quantitative analysis of the social behaviors showed that the 3 KO monkeys (from 2 species, excluding T105) were scored with a tendency toward lower social hierarchy (Fig. 3D) and lower social interaction time (Fig. 3E and F, $P = 0.052$). Moreover, they also exhibited significantly

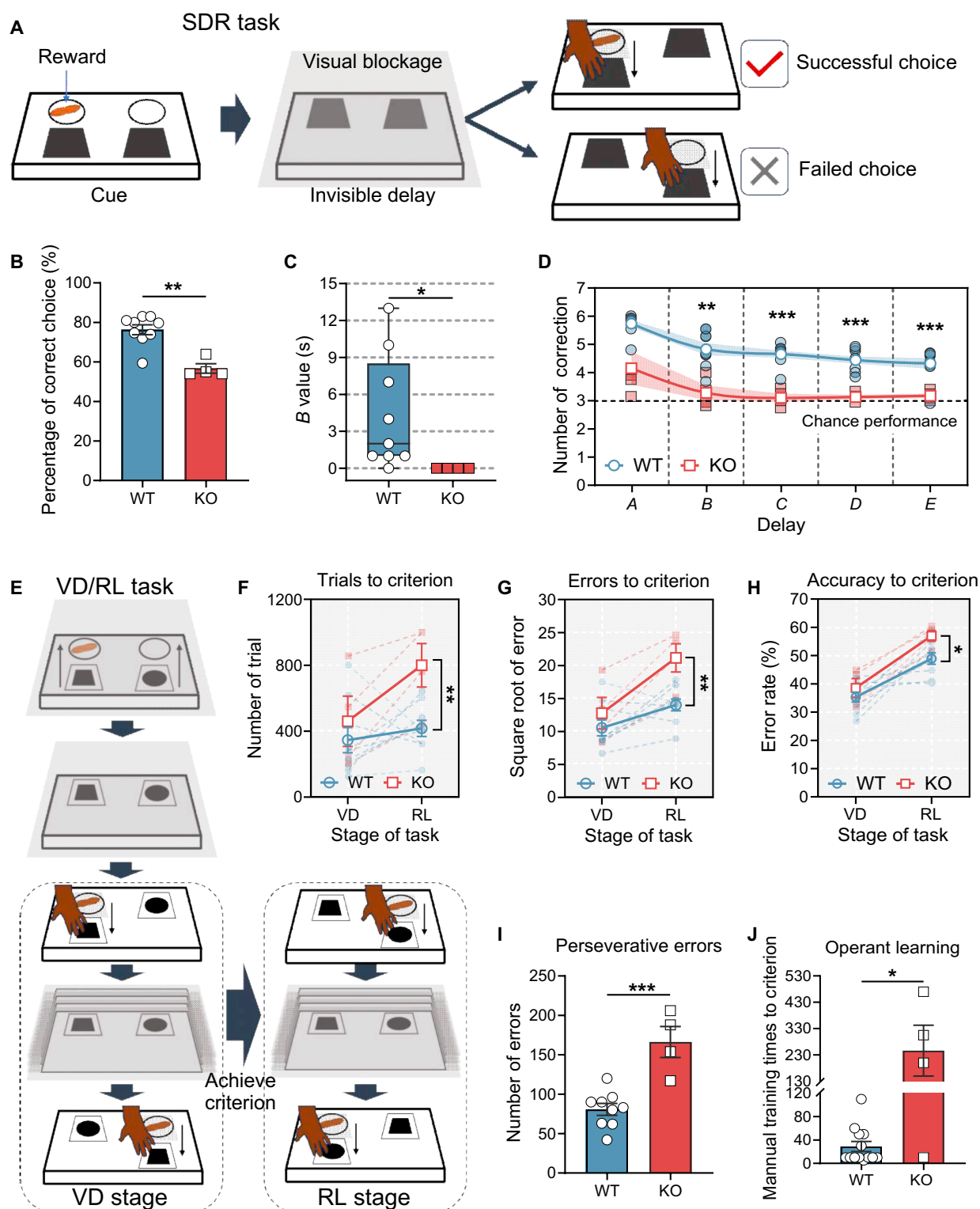


Fig. 2. Impairment of cognitive functions in the giant *ANK2* knockout (KO) monkeys. (A) Schematic diagram of spatial delayed response (SDR) task using a Wisconsin general test apparatus. (B to D) Monkey performance in working memory assessed by the SDR task. The KO monkeys showed a lower percentage of correct choice when $B = 2$ s (B), lower B delay (C), and less mean correctness across 5 delays (D). (E) Schematic diagram of visual discrimination and reversal learning (VD/RL) task using a Wisconsin general test apparatus. (F to I) Monkey performance in cognitive flexibility assessed by the VD/RL task. In the stimulus discrimination stage, KO monkeys had similar numbers of training trials (F), error numbers (G) and mean error rate (H) as the WT controls, indicating normal associative learning. However, they showed impaired cognitive flexibility in the RL task, requiring more training trials (F), making more errors (G and I), and achieving lower accuracy (H). (J) Monkey performance in the operant learning (OL) task. The KO monkeys had more manual training trials to reach criterion, indicating defects in OL. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$, KO versus WT; Mann-Whitney U test for (B) and (J); one-sample t test for (C); linear mixed-effects model (LMM) with Fisher's least significant difference (LSD) multiple comparisons for (D) to (H); Student's t tests for (I). Data were presented as means $\pm$ SEM. KO group, $n = 4$ (including 2 rhesus and 2 cynomolgus monkeys); WT group, $n = 9$ for (B) to (I) (3 rhesus and 6 cynomolgus monkeys) and $n = 13$ for (J) (10 rhesus and 3 cynomolgus monkeys).

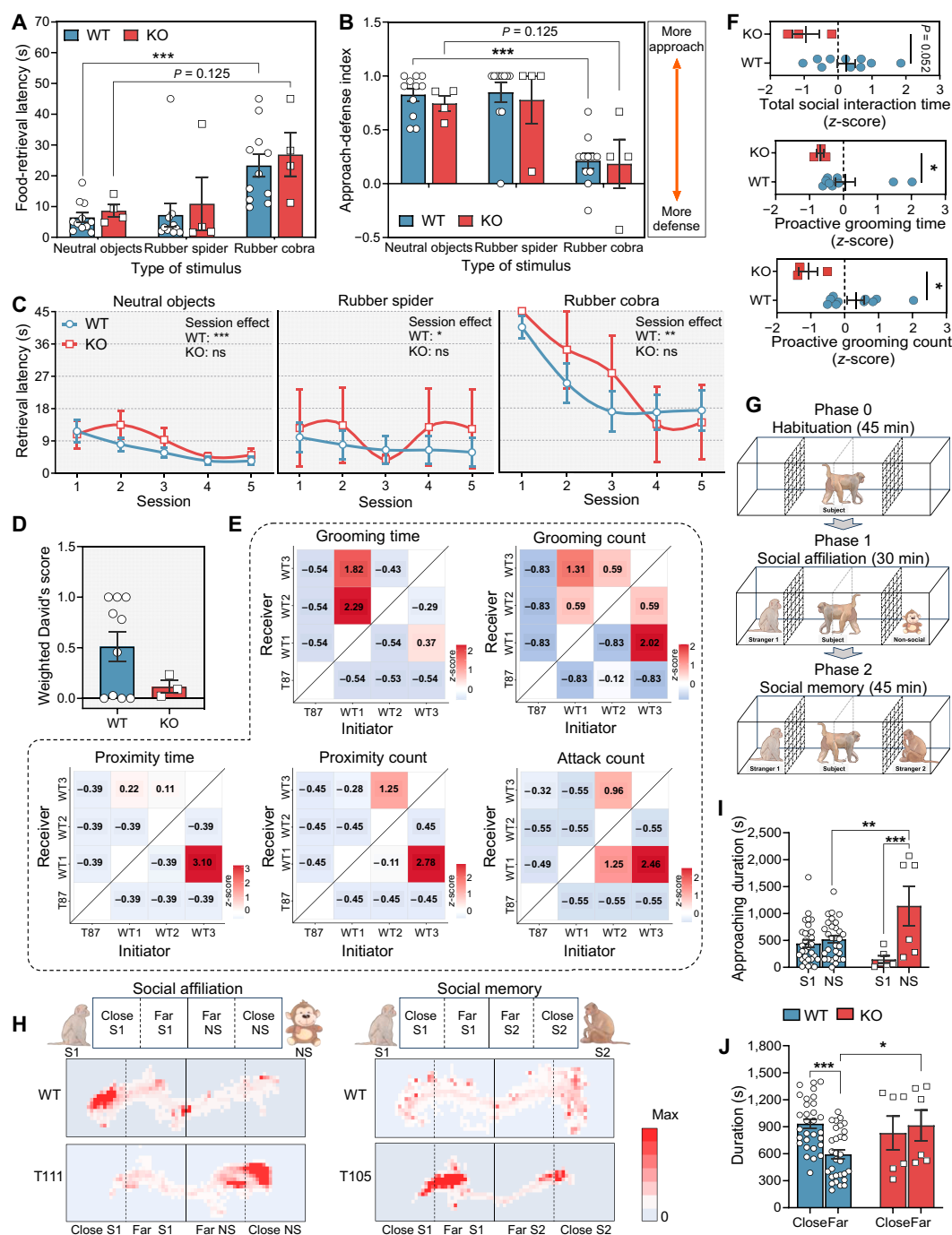


Fig. 3. Abnormal emotional responses and social interaction in the giant *ANK2* knockout (KO) monkeys. (A) Average food-retrieval latency in the emotional response (ER) test. The wild-type (WT) monkeys, but not KO monkeys, displayed significantly longer latency to retrieve food when facing a cobra toy than neutral objects. (B) Behaviors during the ER test scored as the approach-defense index. The WT monkeys, but not KO monkeys, had significantly lower scores when facing a cobra toy than neutral objects. (C) Line plots showing the average food-retrieval latency for each stimulus across 5 sessions. The 2 groups exhibited similar retrieval latency. Unlike KO monkeys, WT monkeys showed significant habituation, with latency decreasing across sessions. (D) Weighted David's score showing the lower tendency of social hierarchy of KO monkeys (with the exclusion of T105) compared to WT monkeys. (E) Heatmaps of z-score normalized dyadic grooming time and count, proximity time and count, and attack count among individual monkeys in one colony. The z-score between a pair of initiator and receiver was defined by a color (red, above the group mean; blue, below the group mean; white, near the group mean). (F) Social behaviors in group-rearing conditions. The KO monkeys showed a lower tendency for total social interaction time and significantly lower proactive grooming time and grooming frequency. (G) Schematic of the 3-chamber social interaction test (SIT) in rhesus monkeys, showing habituation (phase 0), social affiliation (phase 1), and social memory (phase 2). (H) Heatmaps of monkeys approaching duration to stranger 1 (S1)/non-social object (NS) in phase 1, and S1/stranger 2 (S2) in phase 2 from the lateral view. (I and J) Quantification of 3-chamber SIT. The KO monkeys preferentially approached the NS object but not S1 in phase 1 (I). The KO monkeys showed increased avoidance (far) of conspecifics relative to WT monkeys in phase 2 (J). * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$, KO versus WT; Wilcoxon matched-pairs signed-rank test for (A) and (B); Friedman test for (C); Student's *t* tests or Mann-Whitney *U* test for (D) and (F); LMM with Fisher's LSD multiple comparisons for (I) and (J). Data were presented as means $\pm$ SEM, except each trial for individual monkey for (I) and (J). The animal numbers varied in (A) to (C): KO, $n = 4$ (including 2 rhesus and 2 cynomolgus monkeys); WT, $n = 11$ (including 5 rhesus and 6 cynomolgus monkeys); (D) to (F): KO, $n = 3$ (including 1 rhesus and 2 cynomolgus monkeys; one KO rhesus monkey [T105] was excluded); WT, $n = 10$ (including 2 rhesus and 8 cynomolgus monkeys); and (G) to (J): KO, $n = 2$ rhesus monkeys; WT, $n = 10$ rhesus monkeys.

lower proactive grooming time ($P = 0.018$) and proactive grooming count (Fig. 3E and F, $P = 0.018$). However, no differences were found in the duration of passive grooming, passive social behaviors, and social withdrawal (Fig. S2H). Consequently, the social interaction deficit appeared specific to proactive behaviors. To further confirm this observation, we performed the 3-chamber SIT in rhesus monkeys (Fig. 3G). In the social affiliation test (phase 1), the 2 KO rhesus monkeys spent significantly more time approaching the nonsocial object (toy monkey) than stranger 1 ($P < 0.001$), and this nonsocial approaching duration was also significantly prolonged compared with that of WT monkeys (Fig. 3H and I, $P = 0.001$). In the social memory test (phase 2), KO monkeys displayed increased social avoidance, spending significantly longer time distant from both strangers 1 and 2, relative to WT monkeys (Fig. 3H and J, $P = 0.021$). No significant difference was observed between approach and avoidance durations toward the 2 conspecifics in KO monkeys, whereas WT monkeys exhibited significantly longer approach than avoidance durations toward both strangers (Fig. 3J, $P < 0.001$). Together, the results suggested that KO monkeys prefer nonsocial objects over conspecifics, which may explain their diminished proactive behaviors in the group.

MRI-based assessment of brain structural and functional changes in giant *ANK2* KO monkeys

Consistent with our previous observation of drastic brain structural change in these KO monkeys at 6, 12, and 24 months of age [11], we found sustained cerebral ventricular enlargement in KO monkeys by 4 years of age, leading to pronounced morphological alterations in the occipital, temporal, and posterior parietal lobes (Fig. 4A). To minimize segmentation errors resulting from these anatomical alterations, we restricted the structural and functional MRI analysis to the frontal lobe (29 regions), the anterior parietal lobe (10 regions), and 6 subcortical regions (Table S3 and Fig. S4).

We first examined cortical gray matter thickness (GMT). KO monkeys of both species showed significantly reduced GMT in 3 frontal regions (area 24a/b: corrected $P < 0.001$, area 24a/b prime: corrected $P = 0.007$, and area 25: corrected $P = 0.033$) compared to WT monkeys. At the uncorrected threshold, area 32 also showed reduced GMT ($P = 0.019$) in KO monkeys (Fig. 4B). In contrast, area 24c showed significantly increased GMT in KO monkeys relative to WT monkeys (corrected $P = 0.007$) (Fig. 4B). No significant differences were observed in parietal regions, either before or after correction. Overall, these GMT analyses demonstrated altered frontal cortical gray matter in KO monkeys, with most false discovery rate (FDR)-corrected regions showing reduced thickness (Fig. 4B and C). To further characterize the changes in gray matter morphology, we analyzed cortical surface area and gray matter volume. These analyses identified several regions showing group differences at the uncorrected threshold, with most regions showing reduced values in KO monkeys (Fig. S5A to D). Among the subcortical regions examined, pallidum volume was significantly reduced in KO monkeys (Fig. 4D, corrected $P = 0.030$). We also examined T1-weighted/T2-weighted-based myelination estimates. KO monkeys exhibited significantly increased myelination in 2 subcortical structures (Fig. 4E, hypothalamus: corrected $P = 0.004$ and pallidum: corrected $P = 0.019$). Additional increases were observed at the uncorrected threshold in the accumbens ($P = 0.036$), putamen ($P = 0.008$), M1 ($P = 0.036$), and medial agranular insular area

(lam)/posteromedial agranular insular area (lapm) ($P = 0.040$), although these did not reach FDR-corrected significance.

Next, we analyzed functional connectivity (FC) based on resting-state functional MRI (rs-fMRI) data using generalized LMMs at both the lobar and regional levels. At the lobar level, KO monkeys exhibited altered intra- and interhemispheric FC patterns (Fig. 4F). Specifically, intrahemispheric FC within the parietal lobe was significantly increased (corrected $P = 0.025$), while intrahemispheric FC between subcortical structures and the frontal lobe was significantly decreased (corrected $P = 0.032$) (Fig. 4F). At the uncorrected threshold, interhemispheric FC within the parietal lobe was increased ($P = 0.025$), whereas interhemispheric FC among subcortical structures ($P = 0.026$) and interhemispheric FC between subcortical structures and the frontal lobe ($P = 0.026$) were reduced (Fig. 4F). At the regional level, 2 parietal regions in KO monkeys, including area 7op (corrected $P = 0.011$) and area 5d (corrected $P = 0.011$), showed significantly increased FC density (FCD) (Fig. 4G and H). Additional differences were observed at the uncorrected threshold, including increased FCD in superior parietal lobule area (PEa) ($P = 0.007$) and reduced FCD in 3 frontal regions—area 24a/b ($P = 0.010$), area 14 ($P = 0.036$), and area 10 ($P = 0.046$)—as well as in the accumbens (Acb; $P = 0.008$) and putamen (Pu; $P = 0.017$) (Fig. 4G and H).

Beyond FC, we further characterized differences in brain network topology between WT and KO monkeys. The KO monkeys presented a significant reduction in the global small-world parameter σ (Fig. 4I, corrected $P = 0.007$), reflecting altered balance between local clustering and long-range connectivity within the analyzed network. Degree centrality (Dc) analyses revealed significant increases in 3 parietal regions, including PEa, area 5d, and area 7op, as well as in one frontal region, area 8A, whereas the putamen showed a significant decrease in Dc (all corrected $P < 0.05$; Fig. S6A to C). Additional Dc changes were observed at the uncorrected threshold in frontal regions, parietal regions, and subcortical structures, including increased Dc in presupplementary motor area (preSMA), dorsal premotor cortex, and areas 1-2, and reduced Dc in area 24a/b, area 3a/b, area 10, area 9, area 46d, area 13, area 24c, and accumbens (Fig. S6A to C). Furthermore, nodal clustering coefficient (NCp) also showed significant group differences (corrected $P < 0.05$) in multiple regions (Fig. S6D to F). Specifically, NCp was significantly increased in several frontal and parietal regions, including SMA, area 3a/b, area 24c prime, and preSMA, whereas area 25 and area 24a/b showed significantly reduced NCp. Additional NCp differences at the uncorrected threshold were observed in frontal, parietal, and subcortical regions, including increased NCp in secondary somatosensory cortex, ventral premotor cortex, hypothalamus, areas 1 and 2, M1, lateral intraparietal area, and area 8B (Fig. S6D). Detailed regional results are provided in Tables S4 to S6.

To assess the behavioral relevance of the regional neuroimaging abnormalities, we performed exploratory brain-behavior correlation analyses using 3 representative behavioral measures and neuroimaging measures that showed significant group differences after FDR correction. Partial Spearman correlations controlling for species and genotype showed that working memory accuracy at $B = 2$ s was positively associated with cortical thickness in area 24a/b prime ($P = 0.015$) and with Dc of the putamen ($P < 0.001$; Fig. S7). No significant associations were found between neuroimaging measures and RL perseverative errors or cobra-minus-neutral retrieval latency. Together,

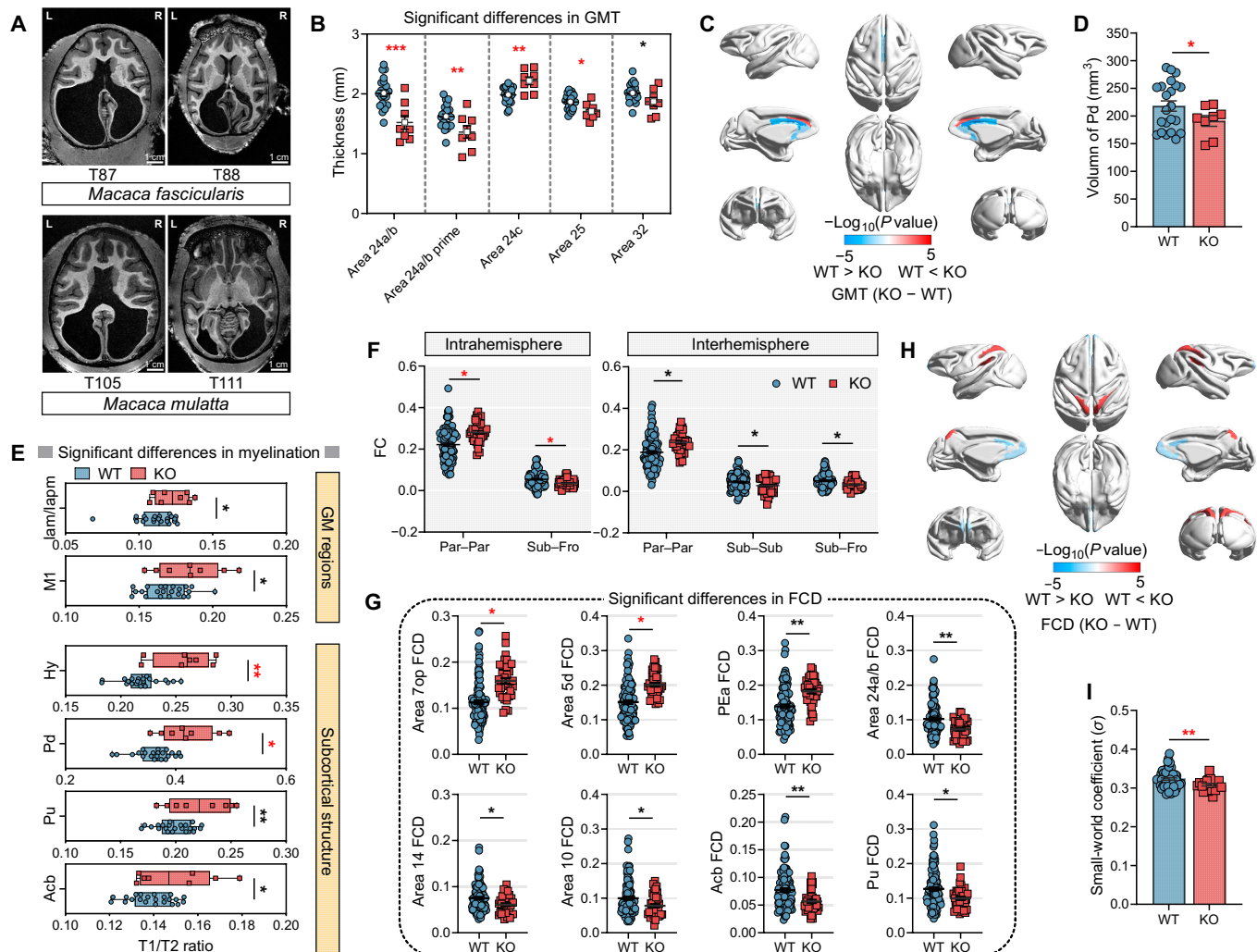


Fig. 4. Abnormal brain structures and functional connectivity (FC) in the giant *ANK2* knockout (KO) monkeys. (A) Representative axial slices of T1-weighted images showing morphological alterations in brain structure for each KO monkey at 4 years old. Please note that the MRI images of KO monkeys T88 and T105 were incorrectly switched in the original Fig. S5 of our earlier publication (Qin et al. [11]). (B) Regions in the frontal and parietal lobes exhibiting differences in gray matter thickness (GMT) between KO and wild-type (WT) monkeys. (C) Mapping of GMT altered regions in (B) onto a monkey brain template. (D) Significant volume difference of the pallidum (Pd) between KO and WT monkeys. (E) Regions showing differences in myelination, calculated from the T1-weighted/T2-weighted ratio between KO and WT monkeys. GM, gray matter; Hy, hypothalamus; Pu, putamen; Acb, accumbens. (F) Lobe pairs showing differences in intra- and interhemispheric FC between KO and WT monkeys. (G) Regions exhibiting differences in FC density (FCD) between KO and WT monkeys. (H) Mapping of altered regions in (H) onto a monkey brain template. (I) Small-world sigma coefficient, showing significant difference between WT and KO monkeys. Regions/lobe pairs surviving false discovery rate (FDR) correction and regions/lobe pairs showing group differences at the uncorrected threshold are displayed. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$, KO versus WT; generalized linear-mixed models. Red asterisks denote FDR-corrected P values; black asterisks denote uncorrected P values. In the box plots in (E), the center line represents the median, the box indicates the interquartile range, and the whiskers extend to 1.5 times the interquartile range to illustrate the full data distribution. Other data were presented as means $\pm$ SEM. KO group, $n = 4$ (including 2 rhesus and 2 cynomolgus monkeys); WT group, $n = 11$ (including 5 rhesus and 6 cynomolgus monkeys). The brain regions were defined in Table S3.

these results indicate that regionally selective cortical structural and subcortical network abnormalities may be associated with the working memory impairment observed in giant *ANK2* KO monkeys.

Physical and hematological parameters in giant *ANK2* KO monkeys

There were no significant differences in physical development (Fig. S8) and echocardiogram indices between the giant *ANK2* KO and WT monkeys, consistent with our previous report [11], except for a lower aortic valve velocity in KO monkeys (Table S7). These observations suggested that KO of giant *ANK2* may

not cause problems with the heart and other organs although previous studies have shown an association of *ANK2* mutations with cardiovascular diseases and other diseases [15–17,36].

In the blood biochemistry test, we found significantly lower levels of total bilirubin ($P = 0.037$), total protein ($P = 0.037$), globulin ($P = 0.007$), blood urea nitrogen ($P = 0.007$), total protein-to-albumin ratio ($P = 0.003$), higher alanine aminotransferase ($P = 0.022$), and albumin-to-globulin ratio ($P = 0.003$) in the KO group ($n = 4$) compared to the age-matched WT group with large sample size ($n = 78$) (Fig. 5A). Moreover, the KO monkey exhibited a significantly lower mean corpuscular volume ($P = 0.002$) and a marginal significance of a higher red blood cell count ($P = 0.080$) when compared to the

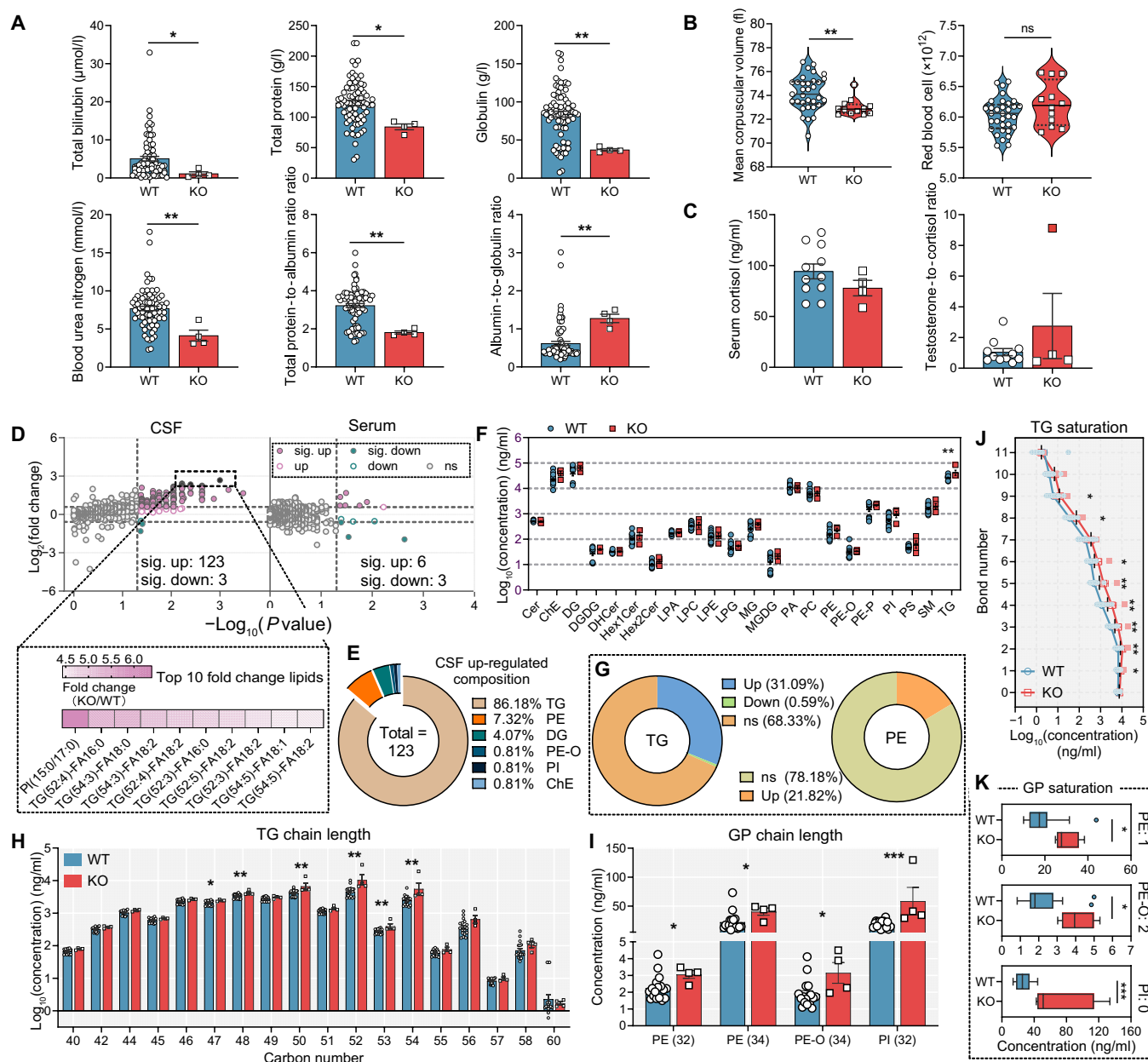


Fig. 5. Altered hematological parameters and lipidomic species in cerebrospinal fluid (CSF) in the giant *ANK2* knockout (KO) and wild-type (WT) monkeys. (A) Comparison of the blood biochemical parameters between KO ($n = 4$, including 2 rhesus and 2 cynomolgus monkeys) and age-matched WT monkeys from both species ($n = 78$, including 58 rhesus and 20 cynomolgus monkeys). (B) Comparisons of mean corpuscular volume and red blood cell between KO ($n = 4$) and WT ($n = 11$, including 5 rhesus and 6 cynomolgus monkeys) groups. The data denoted a single measure for an individual, total 3 measurements during the development. (C) Comparison of cortisol and testosterone-to-cortisol ratio between KO ($n = 4$) and WT ($n = 11$) monkeys. The KO monkey (T105) showing an outlier value was marked in red. (D) Changes in lipid species in CSF and serum between KO ($n = 4$) and age-matched WT ($n = 17$, including 11 rhesus and 6 cynomolgus monkeys) groups. The top 10 fold change species (including 1 phosphatidylinositol [PI] and 9 triglycerides [TG]) were denoted as black points and were zoomed in the black dashed box. At the uncorrected threshold, significant up-regulation (sig. up) and down-regulation (sig. down) were defined by pink and green dot respectively, while up-regulation (up), down-regulation (down), no significance (ns) were defined by pink, green, and black circle respectively. (E) Composition of increased lipid species in CSF between WT and KO groups, showing over 85% of lipid species were TG. (F) Concentration of each lipid class between KO and WT monkeys. (G) Percentage of changed species in TG class and phosphatidylethanolamine (PE) class between KO and WT monkeys. (H) Comparison of concentrations of TGs with different carbon chain lengths between KO and WT monkeys. (I) Comparison of concentrations of lipid species in glycerophospholipids (GP) with different chain lengths. Higher concentrations of PE with 32 and 34 carbons, alkyl-PE (PE-O) with 34 carbons, and PI with 32 carbons were observed in KO monkeys compared to WT monkeys. The carbons numbers were in parentheses. (J) Increased unsaturated TG in KO monkeys compared to WT monkeys. The y axis presented the number of carbon-carbon double bond, with 0 referring to saturated TG and 1 to 11 representing unsaturated TGs. (K) Box plot showing the saturation status of GP. The PE with 1 double bond (PE: 1), PE-O with 2 double bonds (PE-O: 2) and saturated PI with no double bond (PI: 0) were increased in KO monkeys compared to WT monkeys. ns, no significant; * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$, KO versus WT, at the uncorrected threshold; Student's t test or Mann-Whitney U test. In the violin plot in (B) and box plot in (K), the center line represents the median, the box indicates the interquartile range, and the whiskers extend to 1.5 times the interquartile range to illustrate the full data distribution. Other data were presented as means $\pm$ SEM.

WT group ($n = 11$) across 3 measures during the development (Fig. 5B). Other hematological parameters with no significant difference were shown in Table S8. The development-related hormone, i.e., cortisol and testosterone, had no significant differences between the 2 groups (Fig. 5C). However, we found that monkey T105 had abnormally elevated testosterone (5.35 ng/ml) and a high testosterone-to-cortisol ratio (9.12) (Fig. 5C), both exceeded the normal range observed in age- and species-matched WT males (testosterone-to-cortisol ratio = 1.22 ± 0.20 , $n = 8$, means $\pm$ SEM). At age 3, it was also larger (body length, 53 cm) and heavier (body weight, 6.08 kg) than male control monkeys (length, 44 ± 0.44 cm; weight, 3.7 ± 0.04 kg; $n = 3$, means $\pm$ SEM). We assumed that these changes may be associated with the abnormal behavior of this monkey (Fig. S2G).

Up-regulated triglyceride and glycerophospholipids in cerebrospinal fluid in giant ANK2 KO monkeys

The lipid metabolism was closely related to brain development, aging, and disorders [37,38]. We therefore performed a targeted lipidomic profiling of cerebrospinal fluid (CSF) and serum using liquid chromatography–mass spectrometry (Fig. S9A). At the uncorrected threshold, whereas few lipid molecules in serum were altered in KO versus WT groups, CSF showed extensive changes (fold change > 1.5 or < 0.67 , $P < 0.05$), prompting our subsequent focus on CSF (Fig. 5D and Table S9). Notably, almost all differential lipids were up-regulated. While a phosphatidylinositol (PI) species showed the highest fold change (Fig. 5D), the vast majority fell under that of glycerolipids where triglyceride (TG) species predominated, and the second most highly changed category were glycerophospholipid (GP) that was mainly composed of phosphatidylethanolamine (PE) (Fig. 5E). Consistently, overall TG concentration was higher in KO monkeys ($P = 0.004$, Fig. 5F) than in WT monkeys, while total glycerolipids and all other lipid categories did not change (Fig. S9B). Note that up-regulation affected a sizable fraction of both TG and PE species (Fig. 5G).

We next examined lipid structural changes, i.e., chain length as carbon number and saturation as double-bond count, where a higher double-bond indicated greater unsaturation. At the uncorrected threshold, KO monkeys showed elevated TG concentrations across most chain lengths, with increases in mid-length species (Fig. 5H, all $P < 0.05$). Moreover, GP species including PE, PI, and alkyl-PE (PE-O) at specific chain lengths were also increased in KO monkeys (Fig. 5I, all $P < 0.05$). Unsaturated TG concentrations were preferentially elevated in moderately unsaturated species, raising the total concentration of unsaturated TG and the unsaturated TG fraction in KO monkeys (Fig. 5J and Fig. S9C, all $P < 0.05$). The membrane fluidity was assessed on the basis of GP-associated indicators, i.e., saturated fractions and unsaturated concentrations of GP, phosphatidylcholine (PC), and PE class (including PE, PE-O, and alkenyl-PE [PE-P]) following a previous study [38]. The saturated fractions were negatively correlated with fluidity, while unsaturated concentrations were positively correlated. However, no significant changes in fluidity were detected between the 2 groups (Fig. S9D). Despite this, specific PE/PE-O species with fewer double bonds and saturated PI were significantly increased in KO monkeys (Fig. 5K, all $P < 0.05$). Collectively, our CSF lipidomic analyses showed that giant ANK2 KO up-regulates TG and GP species in monkey CSF, potentially linking KO of giant ANK2 to the disruption of brain lipid homeostasis in monkeys.

Dysregulated transcriptomic immune profiling in peripheral blood in giant ANK2 KO monkeys

To further investigate the physiological impact of giant ANK2 ablation, we compared peripheral blood transcriptomic profiles between KO and WT monkeys. Among genes with over 2-fold expressional changes in KO cynomolgus monkeys (1936 genes) and KO rhesus monkeys (524 genes) ($|\log_2$ fold change| > 1 and $P < 0.05$), a number of genes related to immune processes were dysregulated at the uncorrected threshold. Gene expression and differential expression patterns (represented by $\log_2$ fold change) were strongly correlated between the 2 monkey species (Fig. S10). The observed differences in the number of differentially expressed genes (DEGs) between species might reflect underlying genomic variability [39] or differential compensatory responses to ANK2 depletion. Genes with important roles in inflammatory regulation, including *TLR5*, *CCL2*, *AUZ*, *TARM1*, and *CLEC4D*, were down-regulated in KO cynomolgus monkeys (Fig. 6A). Key molecules involved in the type I interferon signaling pathway, such as *IFI6*, *IFIT3*, *OAS1*, *OASL*, *RSAD2*, and *MX1* were also remarkably down-regulated in KO rhesus monkeys (Fig. 6B). DEGs in these 2 groups of KO monkeys were enriched in immune-process-related pathways, especially for pathways related to cytokine production (GO:0001819, positive regulation of cytokine production; Fig. 6C). Among commonly dysregulated genes shared by both KO monkey species, a large proportion of genes were related to the immune process, including *TLR5*, *CLEC1A*, *ATF3*, *GPR84*, *ENTPD1*, *ADGRE1*, *KLRC1*, *CXCR6*, and *NCR1* (Fig. 6D). These results were consistent with immune system abnormalities and cytokine alterations in patients with ASD reported by previous studies [40]. Furthermore, the mRNA expression levels of immune-related genes and the protein levels of cytokines and other immune-related proteins were significantly altered in the peripheral blood of patients with ASD [40,41]. Future validation experiments should be performed to determine whether these DEGs and enriched pathways participate in brain pathology caused by the giant ANK2 KO.

Discussion

The ANK2 gene is highly conserved, with its protein AnkB expressed in organs, such as the brain, heart, skeletal muscle, and thymus [16], and its mutations associated with many diseases, especially NDDs, including ASD [17,36,42]. In our previous study, we generated rhesus and cynomolgus monkeys deficient of the giant ANK2 isoform AnkB-440 [11]. These KO monkeys from both species exhibited massive brain volume loss at early ages (6 months, 1 year, and 2 years) [11] and sustained this loss up to 4 years of age or older. We proposed that this structural defect would cause neural impairments that emerge with development and aging, rather than in infancy [11]. In this study, longitudinal studies of the giant ANK2 KO monkeys revealed hyperactivity, cognitive deficits (in working memory, flexibility, and OL), and disrupted social interactions, aligning with human ANK2 mutation phenotypes [43]. However, the hyperactivity contrasted with the mice carrying an analogous AnkB-440 mutation, which showed reduced activity [10], suggesting species-specific differences. In addition, the giant ANK2 KO monkeys displayed emotional dysregulation, and adolescent KO monkeys exhibited social deficits and a disrupted social hierarchy or affiliation in the colony, probably due to increased avoidance rather than approaching behavior, aligning with prenatally valproate-induced ASD-like

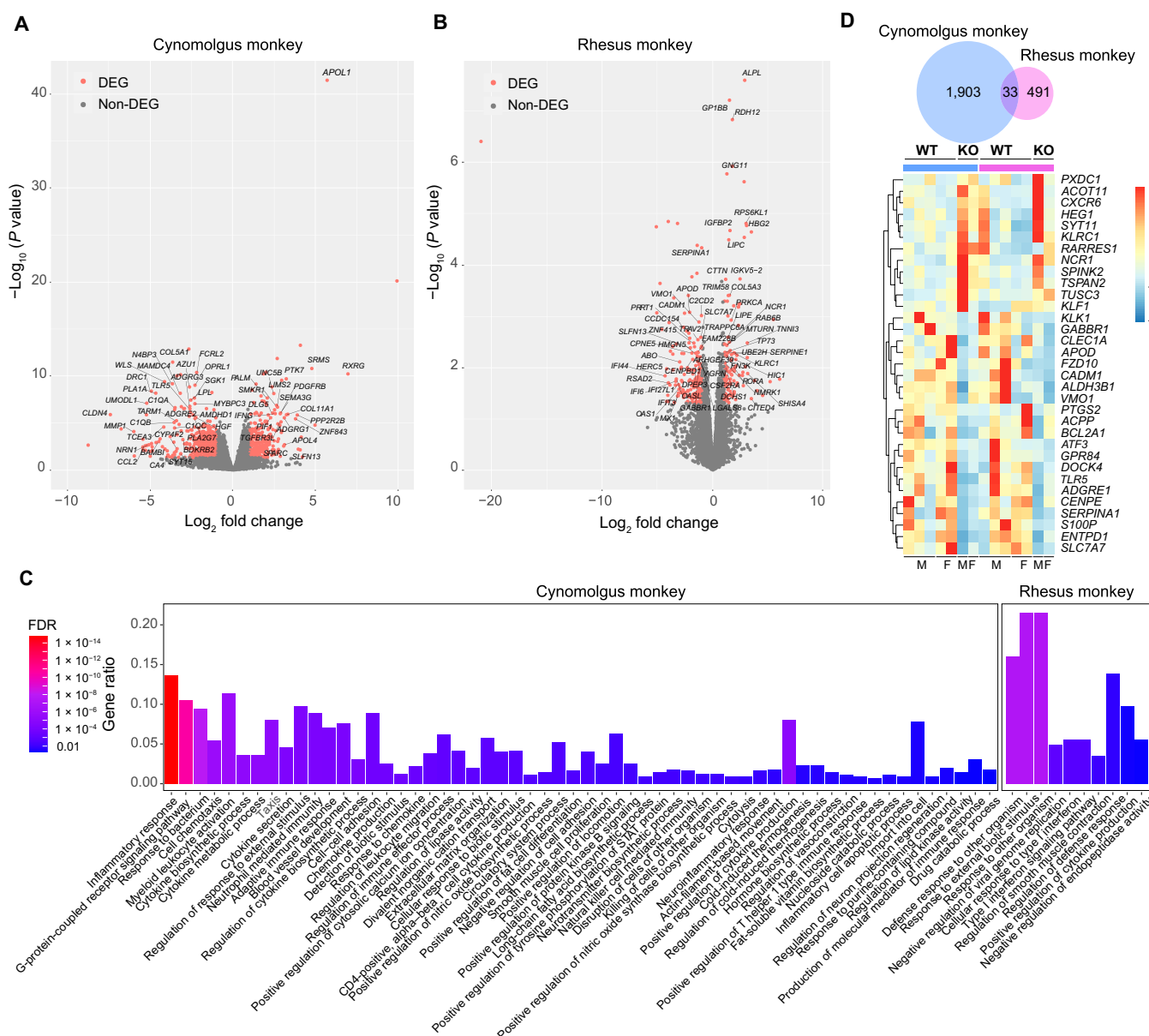


Fig. 6. Transcriptome analysis revealed dysregulated immune process in the giant ANK2-KO (KO) monkeys. (A and B) Top differentially expressed genes (DEGs) in KO monkeys compared to wild-type (WT) monkeys. (C) Enriched biological pathways of DEGs between KO and WT monkeys. Gene ontology, biological process, FDR < 0.05. (D) Heatmap of 33 shared DEGs between cynomolgus and rhesus monkeys. The DEGs between KO and WT groups were defined by $|\log_2 \text{FC}| > 1$ and $P < 0.05$. Rhesus group, 2 KO and 5 WT monkeys; cynomolgus group, 2 KO and 5 WT monkeys. M, male; F, female; STAT, signal transducers and activators of transcription.

rats and schizophrenia [44,45]. Although these KO monkeys displayed social deficits, they did not exhibit overt stereotypical behaviors; thus, we cannot conclude that they fully recapitulate ASD. Nevertheless, they may model specific symptomatic features of NDDs. Of note, these behavioral abnormalities were not present during infancy or juvenility [11] but emerged in KO monkeys as social hierarchy structures matured. Consequently, detecting social behavioral differences in infant monkeys is inherently challenging.

Giant ANK2 KO monkeys showed persistent cerebral ventricular enlargement, resulting in morphological alterations in the occipital, temporal, and posterior parietal lobes, as well as some subcortical regions. Outside these regions of structural change, they exhibited reduced GMT in several frontal cortical

regions, particularly within cingulate areas, which may be associated with impairments in working memory and cognitive flexibility found in this study. Notably, difficulty in learning an operant task mirrored intellectual disabilities observed in ANK2 mutation patients [43]. Moreover, rs-fMRI revealed significantly increased FCD in parietal areas, with additional reductions in frontal and subcortical regions observed at the uncorrected threshold, indicating altered regional importance within the analyzed network. Network integration and segregation were also disrupted, characterized by a significantly reduced small-world coefficient and significant region-specific changes in NCp, with additional NCp differences observed at the uncorrected threshold, which may contribute to the observed behavioral abnormalities [46,47]. Importantly, the regional neuroimaging

abnormalities showed behavioral relevance: Poorer working memory performance was associated with reduced cortical thickness in area 24a/b prime and lower Dc of the putamen. These findings provide evidence linking regionally selective brain abnormalities to the cognitive phenotype of giant *ANK2* depletion, although the exploratory nature and small sample size of the correlation analyses warrant validation in larger cohorts.

Intriguingly, in mice, homozygous deletion of *ANK2* restricted to cortical and hippocampal excitatory neurons results in ASD-like behaviors and leads to juvenile seizure-related mortality [48]. In human patients, *ANK2* mutations have been associated with epilepsy [43,49]. Moreover, the *ANK2* variants have been linked to an inherited disorder known as “AnkB syndrome”, characterized by a range of cardiac arrhythmias and cardiomyopathy [15,16]. We observed no epilepsy or cardiac dysfunction in the giant *ANK2* KO monkeys during the continual investigation up to 7 years of age and no elevated neuronal synchronies as seen in epileptic mice [8]. However, the disrupted small-world coefficient in KO monkeys aligned with the findings in childhood absence epilepsy syndrome [50]. Long-term monitoring of cardiac function in these KO monkeys is warranted, particularly at key developmental stages such as middle and old ages. In addition, the KO monkeys with cerebral ventricular enlargement, a feature previously reported in patients with schizophrenia [51], exhibited significantly higher movement speed. This was compatible with the positive symptoms observed in schizophrenia [52]. Future studies could investigate whether these monkeys also display impaired sensory gating and auditory deficits, a well-established clinical hallmark of the psychiatric disorder, to confirm whether these KO monkeys had schizophrenia-like symptoms.

Consistent with our previous study [11], most physical indicators showed no significant differences between KO and WT monkeys. Although there were no significant intergroup differences in body weight across development, 2 outlier individuals deserve attention. One male KO rhesus monkey (T105) exhibited social isolation, necessitating separate housing for its welfare. It showed abnormally elevated testosterone, which is related to aggression and may contribute to ASD [53], and a larger body size relative to age- and species-matched WT monkeys. This particular case of excessive development might represent an alternate NDD manifestation, suggesting a link between abnormal hormone profiles and alterations in behavior and physical growth [54]. However, it remains unclear whether the behavioral alterations in this monkey are associated with such hormonal abnormalities or the gene deletion. Probably, these 2 factors may interact with each other, and further experiments are required to verify their causal relationship. On the contrary, one dwarf female cynomolgus KO monkey (T88) exhibited consistently low body weight (below 2.75 kg) from infancy through adulthood and reduced blood glucose levels (fasting, 2.4 mmol/l; 1 h after feeding, 2.6 mmol/l). Given that the absence of CRISPR/Cas9 off-target effects has been confirmed in our previous study [11], this observation suggested that giant *ANK2* KO may cause complex, variable physiological outcomes, potentially influenced by KO efficiency, gender-dependent response to gene editing, and species-specific factors, although a small fraction of genes (3.7%) showed differential expression between the 2 species [39].

Compared with age-matched WT monkeys, the giant *ANK2* KO monkeys exhibited several alternations in hematological and biochemical parameters, suggesting that the giant *ANK2*

KO may exert broad effects on monkey's physiological processes. Furthermore, our data showed that *ANK2* KO monkeys have elevated CSF levels of TG and GP compared to controls, while blood lipid levels remain unchanged. These KO animals also exhibited cognitive deficits. Although regional neuroimaging abnormalities correlated with behavioral changes, the co-occurrence of CSF lipid alterations and cognitive impairment was well documented. This association was supported by previous findings in both humans and rodents [55,56] and was corroborated by clinical evidence identifying TG as strongly associated with cognitive decline in Alzheimer's disease [57] and in aging individuals with cognitive dysfunction [58]. Furthermore, the concurrent increase in diacylglycerols and PI (15:0/17:0) may point to aberrant PI signaling in the brain following giant *ANK2* deletion, a pathway recently implicated in neurodevelopment [59]. However, the current data are limited at the uncorrected threshold due to the small sample size and do not establish a causal link nor elucidate the underlying mechanisms. Whether and how these lipid changes contribute to the cognitive deficits remain open questions that necessitate further investigation. For example, it would be valuable to determine whether the elevated TG/phosphatidylglycerol species are localized to specific cognition-related brain regions and to explore their potential connections to excitatory/inhibitory transmission via pyramidal cells and interneurons, once brain tissues or organoids become available.

Peripheral blood transcriptome analysis suggested dysregulated immune-related genes (e.g., *TLR5*, *CCL2*, *AUZ*, *TARM1*, and *CLEC4D*) and associated pathways in giant *ANK2* KO monkeys. Whether and how these molecules contribute to the pathological mechanisms underlying NDD- or ASD-like behaviors associated with *ANK2* mutations await further studies using in vivo targeted gene editing or pharmacological manipulation. One interesting question arising from these data is why more DEGs were observed in cynomolgus monkeys compared to rhesus monkeys following giant *ANK2* KO. Focused study with large cohorts of KO animals would help clarify this species-specific difference.

This study has several limitations. First, the small cohort of giant *ANK2*-deficient monkeys necessitates validation with a larger sample. We are currently expanding the breeding of these animals to reduce variability and obtain more robust insights into the functional alterations resulting from giant *ANK2* KO. Second, our MRI and rs-fMRI analyses were necessarily confined to anatomically reliable frontal, anterior parietal, and selected subcortical regions. The observed structural, FC, and graph theoretical alterations characterize abnormalities within this retained frontoparietal-subcortical network, while their generalizability to the excluded regions and whole-brain network organization remains to be established. The lack of brain tissues at different developmental stages prevented molecular/cellular analyses from examining neuronal changes. The expanded cohort and establishment of brain organoids combining with emerging technologies on single-cell sequencing, such as artificial-intelligence-based spatial cellular profiling and single-cell-specific causal network [60,61], may provide more insights into dynamical processes of brain loss and its underlying mechanism. Third, although lipidomic and transcriptomic analyses suggested alterations in metabolic and immune pathways associated with *ANK2* depletion, these data remain to be further validated, and further functional validations are necessary to elucidate the molecular mechanisms underlying these observed changes.

In summary, we conducted a longitudinal study of monkeys deficient in giant *ANK2*, establishing a comprehensive life-span timeline of behavioral, neuroimaging, and physiological changes in these KO monkeys. Extending our earlier observations from infancy [11], these KO monkeys developed progressive behavioral abnormalities with age, including cognitive, emotional, and social deficits. They also exhibited alternations in brain structure and function, alongside dysregulated lipidomic and immune-related transcriptomic profiles. These findings suggest that giant *ANK2* depletion in NHPs recapitulates key phenotypic features of human NDDs, offering cross-species insights into brain functional development and distinct dimensions of disease pathogenesis, and can thereby aid future mechanistic and therapeutic research.

Materials and Methods

For details about the materials and methods, please see the Supplementary Materials.

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Competing interests: The authors declare that they have no competing interests.

Data Availability

The data are freely available upon request.

Supplementary Materials

Materials and Methods
Figs. S1 to S10
Tables S1 to S9
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Longitudinal Characterization of Giant *ANK2*-Depleted Monkeys Suggests Neurodevelopmental-Disorder-Like Phenotypes

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The *ANK2* gene mutations are marked risk factors for autism spectrum disorder, one of the neurodevelopmental disorders (NDDs) that often extends into adulthood and has a complex etiology involving genetic and environmental factors. *ANK2* encodes 2 major isoforms, AnkB-220 (220-kDa isoform of ankyrin-B) and giant AnkB-440. We previously generated a targeted knockout (KO) of giant AnkB-440 in 2 cynomolgus and 2 rhesus monkeys. While no autism-spectrum-disorder-like phenotypes were observed during infancy, we found marked brain volume loss. In this study, we conducted a longitudinal multimodal study in these giant *ANK2* KO monkeys during adolescent and young adulthood. Behavioral results from these giant *ANK2* KO monkeys revealed increased locomotor activity, deficient cognition (including working memory, cognitive flexibility, and operant lever-press learning), and impaired emotional regulation and social interaction. Furthermore, the giant *ANK2* KO monkeys exhibited persistent structural and functional brain abnormalities, up-regulation of brain triglyceride and glycerophospholipids, and peripheral blood transcriptome signatures of immune dysregulation, which may be related to their behavioral alternations. These observations suggest that giant *ANK2* depletion in non-human primates phenocopies aspects of behavioral, neural, and molecular features characteristic of NDDs. This study provides an in-depth exploratory longitudinal characterization of giant *ANK2* functions in primate brains, which may contribute to translational research on NDDs.

Image

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Online Supplementary Materials

Longitudinal characterization of giant *ANK2*-depleted monkeys suggests neurodevelopmental disorder-like phenotypes

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The file includes:

Supplementary Materials and Methods

Supplementary Fig. S1 to S10

Supplementary Tables S1 to S9

Materials and Methods

Animals

A total of four monkeys with giant *ANK2* knockout (KO) (two *Macaca mulatta* (T105, male; T111, female) and two *Macaca fascicularis* (T87, male; T88, female), of which the detailed construction methodology and validation data were first reported in our previous study [11], were used in current study. Eleven age-, gender-, species - matched wild-type (WT) healthy controls (five *Macaca mulatta*, two females and three males; six *Macaca fascicularis*, three females and three males) were mainly included, for social interaction test, more wild-type monkeys were used for controls. For the hematological and lipidomics study, a large sample size of age-, gender-, species(part)-matched WT individuals were used in the same batch of measurement, and the related sample information was listed in the respective figure legends. All animals were housed under standardized conditions with 60% humidity, a temperature of 21 ± 5 °C, and a 12-h light/dark cycle (light on at 7:00 AM). The monkeys received chow, fresh fruits, vegetables, and water *ad libitum*, with daily veterinary care. All procedures complied with the guidelines approved by the Institutional Animal Care and Use Committee (IACUC 20004) of the Kunming Institute of Zoology (KIZ), Chinese Academy of Sciences (CAS), with efforts made to minimize suffering and the number of animals.

Experimental design

We conducted a longitudinal measurement of behavior, neuroimaging, physiology, lipidomics, and peripheral blood RNAseq in the giant *ANK2* KO monkeys, and age-, gender, species (part)-matched WT monkeys (Fig. S1). KO and WT animals of the same species were compared unless otherwise stated. A series of behavioral tests was administered at appropriate ages as follows: The spatial delayed response (SDR) and visual discrimination/reversal learning (VD/RL) tasks [35, 62] were conducted when the monkeys were between 2.3 and 3.4 years old. The emotional response (ER) test [63] was performed at 3.5 years of age. Social hierarchy and interaction [35, 64] were assessed at 5.5 years of age, after the monkeys had been housed in three separate colonies without intervention for one year. Deep learning-based behavioral analysis, home-cage activity and sleep, manual dexterity, neurological function, exploratory behavior tests, the operant learning (OL) task and three-chamber social interaction test

(SIT) were performed between 6 and 7 years of age, following the methods described in our recent studies [34, 35, 65-67]. The monkeys had a magnetic resonance imaging (MRI) test at an age of 4.2 years, and periodically physical and physiological, lipidomics and peripheral blood RNAseq measurements were taken from 1 to 7 years of age.

Behavioral tests

To minimize training variability across individuals, all behavioral testing was conducted using a unified, criterion-based training framework. Monkeys in both groups were trained under identical training schedules, including a fixed number of trials per session, and were required to reach predefined performance criteria before proceeding to the subsequent stages or formal testing. All tasks were performed by the same experienced experimenter.

Measurement of neurological function. The neurological function was scored using a behavioral scale developed in our previous reports [66, 67]. The total score of the scale was 50 points, with 16 points assigned to the motor system, 9 points to skeletal muscle coordination, and 25 points to the sensory system. A score of 0 corresponded to normal behaviors, whereas higher scores represented severe neurological dysfunction. Two well-trained observers conducted the scoring. Monkeys from both species (rhesus and cynomolgus) were measured.

Measurement of home-cage activity and sleep. To evaluate locomotive activity in the two monkey species, an actigraphy device (ActiGraph GT9X Link, ActiGraph LLC, USA) [34, 35] was mounted on each animal's collar. Home-cage activity and sleep were monitored continuously over 11 days, with the first day excluded for acclimation and the subsequent nine days used for analysis. The sleep period was defined as 19:00 to 7:00. Data were sampled at 30 Hz with 60-second epochs [35] and processed using ActiLife 6 software (ActiGraph LLC) to convert raw triaxial acceleration and rotation into analyzable metrics. Average vector magnitude counts (VMC) quantifying the daily activity and sleep parameters (including total sleep time and sleep efficiency) were measured and processed as previously described [35]. Monkeys from both

species were measured.

Exploratory behavior test. A laser-pursuit test was employed to assess monkey exploration behavior [35]. Briefly, a familiar experimenter stood 1 meter away from the home cage and presented a neutral icon to the monkey using three different patterns: rotating the icon clockwise around the cage, rotating it counterclockwise (each with a 5-second cycle), and shaking it near the monkey (within a 5-10 cm range). The duration and frequency of the monkey's responses including mouth licking, hand touching, and visually tracking the icon with head/body movement, were recorded and analyzed. Monkeys from both species were tested.

Measurement of manual dexterity. A vertical slot task which required the animals to retrieve a $7 \times 7 \times 13$ mm hawthorn strip inside the slot was conducted to measure the manual dexterity of monkeys' preferred hands [65, 66]. The task was performed 20 trials per session and each monkey was tested in 5 sessions. A camera (HDR-CS405, Sony, Japan) recorded the entire procedure for subsequent frame-by-frame analysis using Observer software (Noldus Information Technology B.V., Netherlands). Before collecting testing data, monkeys were trained to complete 20 trials within 20 minutes for three days, so as to adapt for a new task. The dexterity was scored based on the average duration of each trial to successfully retrieve, the percentage of successful retrievals, and the percentage of precision grasp (opposition of the thumb and the index finger). Monkeys from both species were tested.

Decomposition of free-moving kinematics and locomotion. The 3D-motion learning toolkit consisted of a multi-view capture system and a machine learning assisted computational framework, which enabled precise unmarkable tracking and quantitative analysis of animal's movements [68], as demonstrated in our recent studies [34, 35]. Briefly, the multi-view system recorded monkey's locomotive behaviors using four RealSense D435 cameras (Intel, USA) positioned 1.1 m from a three-sided transparent 100 cm^3 testing cage, ensuring full coverage. Cameras sampled 30 fps footage (848×480 pixels) after the monkey was transferred into the cage. The computational framework was integrated into the BehaviorAtlas NHP software (v1.01, Guangdong BayONE Scientific Co., Ltd., China). A well-trained

model was generated by labeling 21 key body parts on the monkeys from extracted 4200 extracted frames based on DeepLabCut methodology (BehaviorAtlas Pose Labeler V1.0.0, Guangdong BayONE Scientific Co., Ltd., China). The model automatically recognized the monkey's 21 body parts in targeted footage and reconstructed the 3D skeleton information of the monkey, from which the 46 kinematic parameters (Table S1) were subsequently extracted. With this information, 14 types of locomotive behaviors were decomposed by supervised classification. The behaviors (Table S2) were categorized as with/without apparent displacement (AD/NAD). Principal component analysis (PCA) was performed on the standardized kinematic parameters. To validate the accuracy of 2D coordinate recognition for individual monkeys, we randomly selected 1,000 images and achieved accuracy rate exceeding 90% across all groups. For three-dimensional trajectories reconstruction, we plotted the 3D coordinates of 21 body landmarks. To assess the performance of spontaneous behavior recognition, we randomly sampled 30 frames from each of 50 datasets, which yielded a recognition success rate of 87.44%. We only tested for the rhesus monkeys (KO, $n = 2$; WT, $n = 5$) because the deep learning-based models were optimized for rhesus monkeys, but not for cynomolgus monkeys. Each monkey underwent two 35-min recording trials with an intertrial interval of a couple of days. Every trial was used as a sample for statistical analysis owing to the limitation of sample size.

Spatial delayed response (SDR) task. The SDR task was performed by using the Wisconsin General Test Apparatus (WGTA) [35]. We grouped all rhesus and cynomolgus monkeys into two groups (KO group, $n = 4$, including two rhesus KO and two cynomolgus KO monkeys; WT group, $n = 9$, including three rhesus and six cynomolgus monkeys, with the exclusion of two rhesus monkeys who refused to perform the task) to maximize the sample size for each group. At first, monkey underwent a period of habituating WGTA and experimenter. The monkey was then trained with $B = 0$ s to learn the procedure, until achieving a criterion (86.7%, 26 correct choices out of 30 trials for consecutive 3 sessions). Subsequently, the assessment began at $B = 2$ s. Then, the B delay increased by 3 s if the monkey achieved the predefined performance threshold, otherwise, the B delay would

decrease by 1 s. The maximum of 10 training sessions were allowed if the monkeys failed to meet the performance criteria. A total of 30 trials per daily session were conducted. The rewards were pseudo-randomly placed in the left or right well across a session. Five delay lengths ($A-E$) were semi-randomly assigned per session: $A = B \times 0$ s (second), $B = B \times 1$ s, $C = B \times 2$ s, $D = B \times 3$ s, $E = B \times 4$ s. The criterion was set as animals would achieve 86.7% (26/30 trials) of correctness for consecutive 3 sessions, in a maximum of 10 training sessions. At first, the monkey was trained with $B = 0$ s to learn the procedure until it achieved the above criterion. Subsequently, the assessment began at $B = 2$ s. Then, the B delay increased by 3 s if the monkey achieved the criterion, otherwise, the B delay would decrease by 1 s. The individual monkey may perform a different maximum B delay. Two WT monkeys were unable to complete the SDR tasks due to their timid personalities. The mean percentages of correct choice at $B = 2$ s, maximum B delay for each monkey, and mean number of corrections at each delay across all trials were calculated to indicate the ability of the monkey's working memory.

Visual discrimination and reversal learning (VD/RL) task. The WGTA-based VD/RL task, which assessed the association learning and cognitive flexibility in NHPs, was conducted following the SDR task [62]. Prior to the VD/RL task, all monkeys underwent an adaptation phase during which they were trained to retrieve rewards from wells covered with two identical cardboard pieces. Each animal was required to complete 40 trials per session per day and to achieve a performance criterion of 87.5% correct responses. In the VD stage, the animal was presented with two different, novel cards and allowed to choose either of them. One was covering a food reward (S+), whereas the other was non-rewarded (S-). By training for 40 trials per daily session, the monkey learned which card was associated with the reward and chose the S+ card to retrieve it, indicating that it was able to distinguish between the two different cards. After the monkey achieved a criterion of 87.5% (35/40 trials) for 3 consecutive sessions, the RL stage commenced by reversing the association between the cards and the reward. The task ended after the monkey achieved an 87.5% correct rate or a maximum of 20 sessions. Percentages of correct choices (chose S+) of each

session, trials to criterion, errors before achieving criterion and errors before consecutively correcting for three trials after reversal were calculated.

Operant learning (OL) task. The monkey was trained to receive a food reward by pressing a lever on a customized operant apparatus (Anilab, Anlai Scientific Instrument (Ningbo) Co., Ltd., China). A successful lever-press triggered the release of a fruit-flavored pill (0.25 g) into a receptacle located 140 cm from the lever. The monkey was initially guided by the experimenter to press the lever for a maximum of 0-30 trials (contingent on individual monkey's learning ability) and then allowed to freely engage with the lever for a one-hour session, with a total of 3 to 6 sessions daily, and a maximum of 50 presses per session. This training course ended after the monkey successfully performed 50 autonomous presses per session for three consecutive sessions. The number of manual training trials before the monkey ended the guidance was recorded. The monkeys were food-restricted before training and provided food to normal nutritional intake after daily training, and monkeys from both species were trained.

Emotional responsiveness (ER) test. We used the procedure adapted from Pujara et al. [63]. Monkeys were pretrained with a 30-trial session per day, in which four neutral “junk” objects were randomly presented one at a time for 3 trials, interleaved with 18 empty-box trials. Training continued until the animal retrieved the reward within ~5 seconds on each trial, ensuring reliable retrieval before proceeding to the later test. Food preference was evaluated by randomly placing two of four reward types (hawthorn candy, peanut, raisin, sweet potato) on the left and right sides of a transparent box (55×20×15 cm) in the WGTA. A total of six pairs of rewards were presented 6 times across 36 trials/session. The monkey's preferred food was identified as the most frequently chosen across 5 sessions. The emotional response (ER) test was then conducted, comprising 5 sessions (each with 10 trials, spaced 2 days apart). Each trial featured a novel neutral object or a negative stimulus (a rubber cobra/spider) placed in the box with a favored reward at the center-top. Stimuli were presented semi-randomly once per session (30s intertrial interval; max 45s exposure). Negative stimuli were excluded from the first 4 trials and consecutive presentations. Food-retrieval latency (from trial start to reward retrieval) and approach/defense behaviors

were scored by video analysis. Approach included forward movement, touching, or eating. Defense included freezing ≥ 3 s, startle, aversion ≥ 3 s, or withdrawal to the back of the cage. Approaching (APP) and defending (DEF) might coexist in the same trial. We calculated the approach-defense index (ADI) to quantify the behaviors following the formula: $ADI = (P_{APP} - P_{DEF}) / (P_{APP} + P_{DEF})$, in which the P_{APP} or P_{DEF} was the proportion of trials presenting approaching or defending, respectively. Monkeys from both species were tested.

Social interaction. After over one year of acclimatization in three social colonies (excluding KO rhesus monkey T105 as noted by the welfare manager), all monkeys were video-recorded for 21 consecutive days. Social interactions between 8:00-12:00 and 13:00-17:00 over eight days were scored by four experts using Observer™ software (Noldus Information Technology B.V., Netherlands). Behaviors were classified as described in our previous study [35], including proactive social (affiliative, grooming), passive social (affiliative, grooming), aggression, or submission. Grooming was defined as initiating and maintaining physical contact ≥ 3 s; affiliative behavior as sitting within arm's length, playing, or hugging ≥ 3 s; aggression as threatening, biting, rearing back and opening mouth, roaring, or slapping; and submission as lip-smacking, grimacing, or escaping. Proactive and passive behaviors were measured by duration, while aggression and submission were counted by frequency. Total social interaction time excluded aggressive and submissive events. Social ranks were calculated using weighted David's scores based on aggression or submission outcomes [65], with higher scores indicating higher rank. Monkeys from both species were tested.

Three-Chamber social interaction test (SIT). The three-chamber SIT was adapted from rodent paradigms [69]. The apparatus had four sequentially connected cages (850×850×850 mm each). Left/right cages were separated from the two central interconnected cages (1 and 2) by grid panels, forming three compartments (Fig. 3F). Two test sets in adjacent quiet rooms served different-sex monkeys, each with a front-facing camera (SONY, 30 fps). Three phases were conducted (Fig. 3F) on only rhesus monkeys due to the limitation of the same-species strangers.

Habituation: Monkey explored central cages for 45 min/session/day, three sessions. Time in cage 1 versus cage 2 was analyzed. The side with the longer duration was defined as the preferred.

Social affiliation: Toy (non-social stimulus) was placed on monkey's preferred side, one unfamiliar monkey (stranger 1, social stimulus) was opposite. Monkey was allowed to stay in central cage 1 and 2 for 30 min/session/day, three sessions.

Social memory: Toy was replaced with a novel unfamiliar monkey (stranger 2). The monkey interacted with stranger 1 and stranger 2 through visible grid panels for 30 min/session/day, three sessions. Unfamiliar stimulus monkeys and test subjects were matched for sex, age and size, and any aggressive individuals were excluded. "Close" was defined as the test monkey's entry into the half-cage zone adjacent to the stimulus, reflecting approach behavior. "Far" was defined as the remaining half of the stimulus-side cage plus the full extent of the opposite adjacent cage, reflecting avoidance behavior (Fig. 3G).

MRI acquisition and data processing

Data acquisition. The two macaque species (*Macaca mulatta* and *Macaca fascicularis*), with both represented in similar numbers in both the KO and WT groups to prevent the results from being dominated by one species. MRI scanning was carried out for KO and WT monkeys at 4.2 years of age, following the procedure described in our previous studies [35, 67]. Prior to scanning, monkeys were premedicated with atropine (0.05 mg/kg, i.m., HeNan RunHong Pharmaceutical Co., Ltd., China) to reduce salivation. After 5 min, anesthesia was induced by hydrochloric acidulated ketamine (Jiangsu Zhongmubeikang Pharmaceutical Company, 10 mg/kg, i.m.) and maintained with propofol (15 mg/kg/h, i.v.) during the scanning. Anesthesia was continuously monitored for the animal's safety and signal stability.

The MRI and rs-fMRI data were acquired by a 3.0T MRI scanner (uMR790, United Imaging Healthcare Co., Ltd., China) with a 12-channel head coil at the KIZ. The T1-weight (T1w) images were acquired using a 3D fast spoiled gradient echo (gre_fsp) sequence: voxel size = 0.5 mm isotropic, echo time (TE) = 5.6 ms, repetition time (TR) = 13.01 ms, flip angle (FA) = 8°. The T2-weight (T2w) images were acquired using an fse_mx sequence: voxel size = 0.5 mm isotropic, TE = 396.48

ms, TR = 3400 ms, FA = 59°. The rs-fMRI images were collected using an echo planar imaging (EPI) sequence (voxel size = 1.5 mm isotropic, TE = 29 ms, TR = 1700 ms, FA = 80°), with each session comprising 500 EPI volumes. Reverse phase encoding data were also acquired for EPI image correction.

Because marked abnormalities in ventricular morphology were present in some ANK2 KO monkeys, several adjacent cortical and subcortical regions exhibited excessive deformation during anatomical registration and atlas-based parcellation. Consequently, morphometric and regional MRI/fMRI analyses were restricted to regions with reliable anatomical correspondence across all subjects. Regions were excluded when their boundaries could not be reliably delineated, when cortical surface placement or subcortical segmentation was unstable, or when atlas alignment was deemed unreliable. As a result, the analyses were focused on frontal regions ($n = 29$), anterior parietal regions ($n = 10$), and six main subcortical regions (e.g., the amygdala) per hemisphere (Table S3). All inclusion and exclusion decisions were made based on visual quality control of anatomical reconstruction, surface/segmentation accuracy, and registration quality, rather than on statistical outcomes. The complete list of retained and excluded regions is provided in Supplementary Table S3, and the MRI/fMRI analysis mask is illustrated in Figure S6.

Structural data and myelination process. Structural data were processed using Analysis of Functional NeuroImages (AFNI) [70], FMRIB Software Library (FSL) [71], Advanced Normalization Tools (ANTs) [72] and FreeSurfer [73]. The procedure for generating gray matter and white matter surfaces, and extracting gray matter volume, thickness, and surface area for each cortical region, as well as volume for subcortical regions was in line with our previous studies [35, 67].

In detail, for each animal, the T1w image was registered to the NIMH Macaque Template, NMT version 2.0 [74]. Skull stripping masks were generated using an in-house neural-network-based procedure and were used to support subsequent processing. T2w images were co-registered to the corresponding T1w images using a rigid-body transformation. A bias correction procedure was applied to enhance contrast by combining the T1 and T2 images.

After bias correction, both T1w and T2w images were further denoised using ANTs [72] then standardized using a linear scaling procedure based on values extracted from two anatomical masks located at the eyes and temporal muscles [75].

Myelination images were then obtained from the T1w/T2w ratio, and the average myelination intensity was calculated in each region by excluding outlier voxels ($> 3SD$).

Cortical surface reconstruction was performed using a FreeSurfer-based pipeline to generate white-matter and pial surfaces. Cortical regions were defined according to the CHARM atlas [74]. For each retained cortical ROI, cortical gray-matter volume, gray-matter thickness, and gray-matter surface area were extracted. Subcortical regions were parcellated using the SARM atlas [76]. Atlas labels were transformed from template space to individual subject space using the inverse registration transformation, and regional volumes were calculated based on voxel counts.

Preprocessing rs-fMRI Data. Rs-fMRI data were preprocessed using AFNI [70]. EPI susceptibility distortion was corrected using reverse phase-encoding images. For each animal, the anatomical image was aligned to the EPI images to minimize interpolation-related changes to the EPI signals and to support subsequent anatomical registration. Slice-timing correction was then applied, followed by motion correction of the EPI volumes using 12 motion parameters. Co-registration to NMT space was achieved through the individual anatomical scans. Large transient signals were attenuated using AFNI 3dDespike. To improve the signal-to-noise ratio, EPI volumes were spatially smoothed with a 2-mm full-width at half-maximum (FWHM) Gaussian kernel. Voxel-wise signal intensities were scaled to yield a mean time-series value of 100 for each voxel. To reduce confounding effects, linear trends, head-motion parameters, and the average time series within the white matter (WM) and CSF masks were regressed out. WM and ventricular CSF nuisance masks were defined in the NMT template space and applied the same masks to both groups. Given the pronounced ventricular enlargement and anatomical distortion observed in the *ANK2* KO monkeys, we used anatomically constrained masks to minimize potential tissue misclassification during nuisance-signal extraction. Specifically, the WM mask was restricted to anatomically preserved frontoparietal white matter, corresponding to the cortical territories retained for rs-fMRI analysis, whereas the CSF mask was confined to ventricular CSF compartments. After resampling to each animal, all masks were visually inspected to confirm correct tissue localization, ensuring that the WM mask remained within white matter and that the CSF mask remained within ventricular CSF compartments. Mean WM and CSF signals were then extracted from these masks and

included as nuisance regressors in the rs-fMRI preprocessing pipeline. No temporal bandpass filter was applied to the BOLD signals because neural information may be present outside the conventional 0.01–0.1 Hz frequency range, including higher-frequency components. Each time series was divided into five segments of 100 time points, and subsequent analyses were conducted separately for each segment.

Functional connectivity (FC). FC was calculated as Pearson correlation coefficients between the mean time series of two brain nodes from 90 regions, resulting in a 90×90 matrix, which was then standardized as Z-scores using Fisher's Z-transformation. The 90 regions (Table S3) were grouped into lobes (frontal, parietal) and subcortical nuclei for each hemisphere, creating a 6×6 connectivity matrix by averaging connectivity values within and between lobes, similar to our previous study [67]. These matrices were further categorized as left intra-hemispheric, right intra-hemispheric, and inter-hemispheric connections. Regional FC was indicated by FC density (FCD), which was calculated as the average of positive connections to the remaining 89 areas, with negative connections set to zero [77].

Graph theory. The graph theory analysis, specifically whole-brain and nodal network analyses, were conducted using the GREYNA toolbox [78]. Whole-brain network was demonstrated by the small-world coefficient (σ), which reflected both global integration and local specialization. Nodal network metrics included the nodal clustering coefficient (NC_p), an indicator of functional segregation, and nodal degree centrality (D_c), which quantifies the number of connections a node has and thus its importance within the network [79]. A range of sparsity thresholds from 0.20 to 0.50 (in 0.05 increments) was applied to ensure accurate estimation. Whole-brain and nodal metrics were computed at each threshold, and the area under the curve (AUC) was calculated for each metric [80].

Brain–behavior correlation analysis. To explore the behavioral relevance of regional neuroimaging abnormalities, three representative behavioral outcomes were selected for correlation testing: working memory accuracy at B = 2 s in the spatial delayed response task, perseverative errors in the reversal learning task, and the cobra-minus-neutral retrieval latency difference in the emotional responsiveness test.

Neuroimaging variables were restricted to measures showing significant group differences after FDR correction. Partial Spearman correlations were performed in animals with available paired behavioral and neuroimaging data, controlling for both

species and genotype, with genotype included to reduce the potential influence of the overall KO-WT group difference on the observed associations.

Physiological, hematological and lipidomics measurements

Body weight, body length, head circumference, upper-limb length, and lower-limb length were recorded for each monkey at six time-points (2.3, 2.6, 2.8, 3.6, 4.4 and 7 years old), as described in our previous study [67]. Rectal temperature and heart rate were recorded simultaneously. Cardiac morphology and functions were determined by M-mode echocardiography using a veterinary ultrasound color doppler system (Mylab ClassC VET, Esaote, Italy) (Table S7). All measurements were conducted under light anesthesia maintained with ketamine hydrochloride (5 mg/kg, i.m., provided by Jiangsu Zhongmu Beikang Pharmaceutical Co. Ltd., China).

The fasting blood was collected from the monkey's femoral vein for assessing hematological and ELISA assays. Briefly, serum was extracted from coagulants tube after centrifuged at 1489 g at 4 °C for 15 min and then was used for measuring blood biochemical parameters via integrated chemistry system (Dimension EXL 200, Siemens, Germany). We combined data for both monkey species together for hematological analysis, considering the physiological similarity between rhesus and cynomolgus monkeys. We expanded the sample size of WT group by 78 age-matched monkeys (58 rhesus and 20 cynomolgus monkeys) from our breeding colonies for blood biochemistry test (BCT) at the same measurement.

The serum cortisol and testosterone levels were measured using the QuicKey Pro macaque Cortisol ELISA Kit (Elabscience Biotechnology, E-OSEL-MK0002) and the monkey testosterone (T) ELISA kit (Shanghai Zhenke Biotechnolog, product No. ZK-12626), respectively, according to the manufacturers' protocols. Each sample was measured in triplicate repetitions. EDTA-K2-anticoagulated whole blood samples were collected for measurement of complete blood count (CBC) at three developmental time points (age of 35, 47 and 53 months) (Table S7) via an automatic animal blood cell analyzer (BC2800-vet, Shenzhen Mindray Bio-Medical Electronics Co., Ltd., China).

We also performed targeted lipidomics analysis for cerebrospinal fluid (CSF) and serum samples from WT and KO monkeys. We pooled rhesus and cynomolgus monkeys into KO ($n = 4$) and age-matched WT animals ($n = 17$, including 11 rhesus

and 6 cynomolgus monkeys) to maximize the sample size of *ANK2* KO genotype and WT controls to reduce individual differences. Serum samples were collected as described above and CSF samples were collected from the interspace between the last two lumbar with a sterilized 22-gauge puncture needle in monkeys under anesthesia with ketamine (10 mg/kg, i.m.). The CSF sample was centrifuged at 4 °C, 3000 rpm for 15 min to separate supernatant, which was then immediately stored at –80 °C until analysis. All the lipids were extracted using the MTBE method, and lipid analyses were based on LC/MS, following the procedure described in our previous study [35]. Briefly, the analyses were performed using a UHPLC system (LC-30AD, Shimadzu) coupled with QTRAP MS (6500+, Sciex) at Shanghai Applied Protein Technology Co., Ltd. The analytes were separated using a HILIC column (Phenomenex, Luna NH2, 2.0 mm × 100 mm, 3 µm) and a C18 column (Phenomenex, Kinetex C18, 2.1 × 100 mm, 2.6 µm). The measured metabolites were quantified using Multi Quant or Analyst software.

Peripheral blood sample collection, RNA extraction, and RNA sequencing

For each monkey, 1 mL of venous blood was collected into EDTA-coated collection tubes. Blood samples were immediately transferred to an RNase-free laboratory and centrifuged at 3000 rpm for 10 min at room temperature to separate plasma. The remaining blood samples were incubated with red blood cell lysis buffer (Tiangen, China, RT122-01) at room temperature for 5 min to remove erythrocytes, followed by centrifugation at 4000 rpm at 4 °C for 10 min. Leucocyte pellets were subjected to total RNA extraction by using the RNAeasy kit (TianGen, co.td.) according to the manufacturer's instructions. The integrity and concentration were tested with NanoDrop biophotometer (Thermo Fisher Scientific).

For each monkey, 1 µg of RNA was used for RNA-sequencing. Sequencing libraries were generated using the NEBNextUltra™ RNA Library Prep kit for Illumina (NEB, USA) following the manufacturer's recommendation and index codes were added to attribute sequence to each sample. The processed final library was sequenced on an Illumina Hiseq 4000 platform, and 150 bp paired-ends reads were generated. Raw reads were trimmed to remove sequencing adapters and low-quality reads using Trimmomatic 0.39 [81]. The clean reads were then aligned to the corresponding reference genome (cynomolgus monkey: *Macaca_fascicularis_5.0*;

rhesus monkeys: Mmul_8.0.1) using HISAT2 [82]. FeatureCounts was used to generate counts for known genes [83] and the differential expression analyses were performed using DESeq2 with gender as a covariate [84]. Given the genetic differences between the two macaque species and inter-individual variability, we employed a relatively lenient threshold for defining differentially expressed genes (DEGs), with genes showing $|\log_2\text{FoldChange}| > 1$ and an uncorrected p value < 0.05 considered as DEGs. Orthologous genes among cynomolgus monkey, rhesus monkey, and human were extracted from the Ensembl database (<http://www.ensembl.org>). Gene Ontology enrichment analysis of target gene sets was analyzed by DAVID 6.8 (<https://david.ncifcrf.gov>) [85]. Enriched terms with an FDR < 0.05 were considered statistically significant.

Statistical analysis

Data were expressed as mean $\pm$ standard error of the mean (S.E.M) and analyzed using the GraphPad Prism v10.50 (GraphPad Software, La Jolla, CA, USA) and Matlab. For all results, p values were uncorrected unless explicitly indicated as corrected p values in the results. After verifying data normality with the Shapiro-Wilk test, appropriate statistical methods were selected. A one-sample t -test was used in the SDR task to assess whether the mean B value in WT monkeys differed from the hypothetical value of $B = 0$ s, for all KO monkeys were $B = 0$ s. The repeated-measures data including the five delay lengths in SDR task, two stages in VD/RL task and three phases in SIT were analyzed using a linear mixed-effects model (LMM), which included genotype and delay, stage or phase as fixed effects. Nonparametric Wilcoxon matched-pairs signed rank test was used for concerning the mean latency, ADI and trials before/after rubber cobra within group, as well as nonparametric Friedman test was used for concerning the latency across five sessions to compare the session effect between group in ER test. For the remaining behavioral and physiological data, normally distributed datasets were analyzed using Student's t tests, whereas non-normally distributed data were analyzed with the nonparametric Mann-Whitney U test, due to non-repeated measurements. Specifically, multiple t -tests with the FDR correction were used for the 3D kinematic parameters. Statistical significance was set at FDR-corrected $p < 0.05$ for 3D kinematic parameters or uncorrected $p < 0.05$ for other behavioral and physiological data.

For the analysis of MRI and fMRI data, we used a unified modeling framework across all brain regions, rather than selecting region-specific model families based on post hoc normality or variance tests. Specifically, generalized linear mixed models (GLMMs) with a Gaussian distribution and identity link function were used for the primary analyses, given that the dependent variables were continuous. Structural MRI and myelination differences were assessed using GLMMs at regional levels, with Hemisphere and Species included as random effects. For the global rs-fMRI metric, GLMMs were performed with Segment and Species as random effects. For lobe- and region-level rs-fMRI metrics (e.g., FC and graph theory metrics), GLMMs included Segment, Hemisphere, and Species as random effects. To improve transparency, we added a statistical summary table (Table S6) for the corrected and uncorrected $p < 0.05$ findings reported in the main and supplementary results.

The relevant distributional assumptions primarily concern the model residuals rather than the raw regional values. Shapiro-Wilk test was conducted to assess normality, and Brown-Forsythe tests were conducted to evaluate homogeneity of variances.

Most findings showed clear evidence of residual normality. For findings whose residuals did not pass the Shapiro-Wilk test, we further inspected residual Q-Q plots, which did not indicate severe departures from the Gaussian mixed-model assumption. Only a small number of FDR-corrected findings showed evidence of variance heterogeneity. We performed additional subject-level Welch-based sensitivity analyses for these findings to validate the results. Specifically, repeated measurements within each animal were first averaged to obtain one subject-level value for each relevant region or metric, and Welch tests were then used to evaluate whether the corresponding effects remained robust under unequal-variance assumptions. The direction of the effects remained unchanged, and all tested effects remained significant or approximately significant in the Welch-based sensitivity analyses, indicating that these findings were robust to unequal-variance assumptions. These results were listed in Table S6.

The FDR correction was applied within each analysis family across the applicable tested regions. Cortical-only MRI measures, such as cortical thickness and surface area, were corrected across the retained cortical regions of interest (ROIs, $n = 39$), while measures applicable to both cortical and subcortical regions were corrected across all retained ROIs ($n = 45$). Lobe-pair analyses were corrected across the tested

lobe pairs ($n = 6$). Given the relatively small sample size, both corrected and uncorrected significant results were reported to provide a comprehensive view.

For the lipidomic analysis, we firstly constructed an orthogonal partial least squares-discriminate analysis (OPLS-DA) model and observed a limited reliability of this multivariate statistical model, validating by extremely low R^2X , R^2Y and Q^2 in the 7-fold cross-validation and permutation tests. Therefore, we screened for significantly altered metabolites through univariate analyses. Significantly different lipid specie between the two groups were identified using the Mann–Whitney U test combined with fold change criteria, owing to the small sample size and large proportion of non-normal distributions. A metabolite was considered significantly different if it met the threshold of uncorrected $p < 0.05$ and had a fold change > 1.5 or $< 2/3$, on account for inter-individual variability. The Mann–Whitney U test was also applied to assess differences in chain length, lipid saturation, as well as comparisons at the category and class levels. Uncorrected $p < 0.05$ was also considered as significantly different.

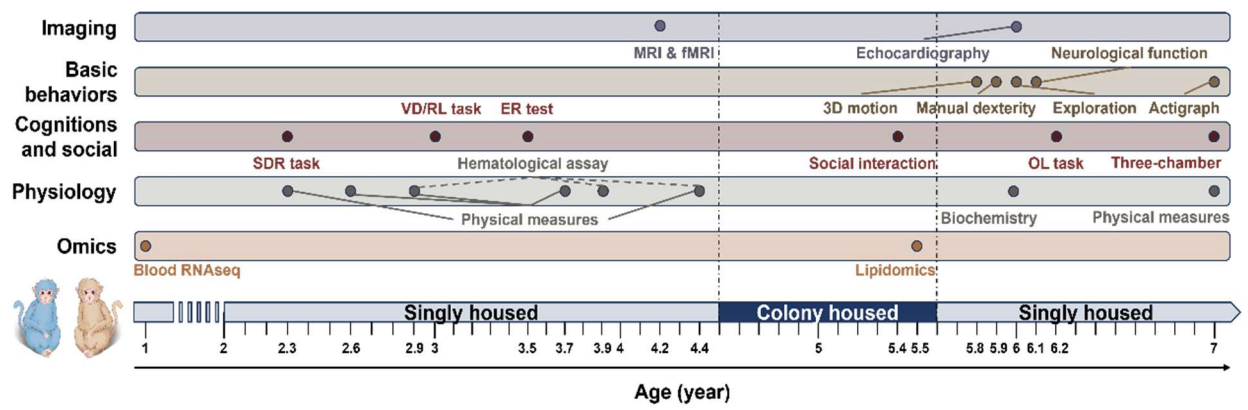


Fig. S1. Study design and timeline for longitudinal measurements.

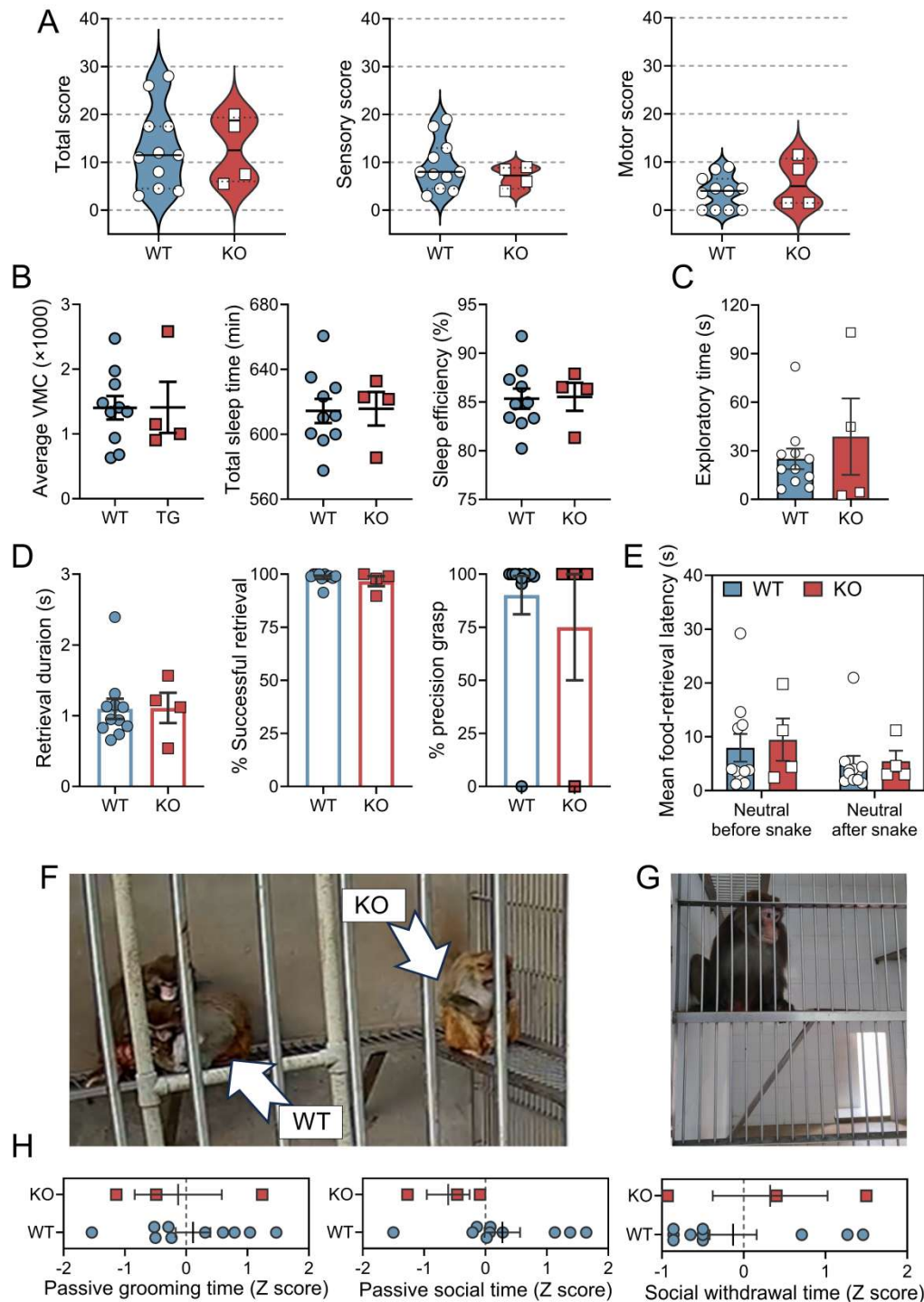


Fig. S2. A profile of behaviors showing no significant difference between the giant *ANK2* knockout (KO) and wild-type (WT) monkeys.

(A) The total score, sensory score and motor score in neurological functions. (B) Average vector magnitude count (VMC) at daytime determined from Actigraph. The sleep pattern was assessed by total sleep time and sleep efficiency. (C) Duration of exploring behaviors. (D) Mean retrieval duration, losing frequency and proportions of

precisely retrieval in the vertical slot task. (E) The mean food-retrieval latency of the trial before or after the rubber snake emerged in the fear-induced emotional response test. (F-G) Representative footages showing the solitary behavior of rhesus KO monkey (T111) and WT monkey (WT) in a group-rearing condition (F), and a male KO rhesus monkey (T105) was reared alone in a single colony unit (G). (H) Passive grooming time, passive social interaction time and duration of social withdrawal during the group-reared condition. Student's *t* test or Mann-Whitney U test for (A-D, H); Wilcoxon matched-pairs signed rank test for (E). In the violin plot in (A) (related to Fig. 1), the center line represents the median, the box indicates the interquartile range (IQR), and the whiskers extend to 1.5 times the IQR to illustrate the full data distribution. Other data were presented as mean $\pm$ S.E.M. KO group, *n* = 4 (including 2 rhesus and 2 cynomolgus monkeys); WT group, *n* = 10-11 (4-5 rhesus monkeys and 6 cynomolgus monkeys; one rhesus WT monkey died from intestinal obstruction at age 7).

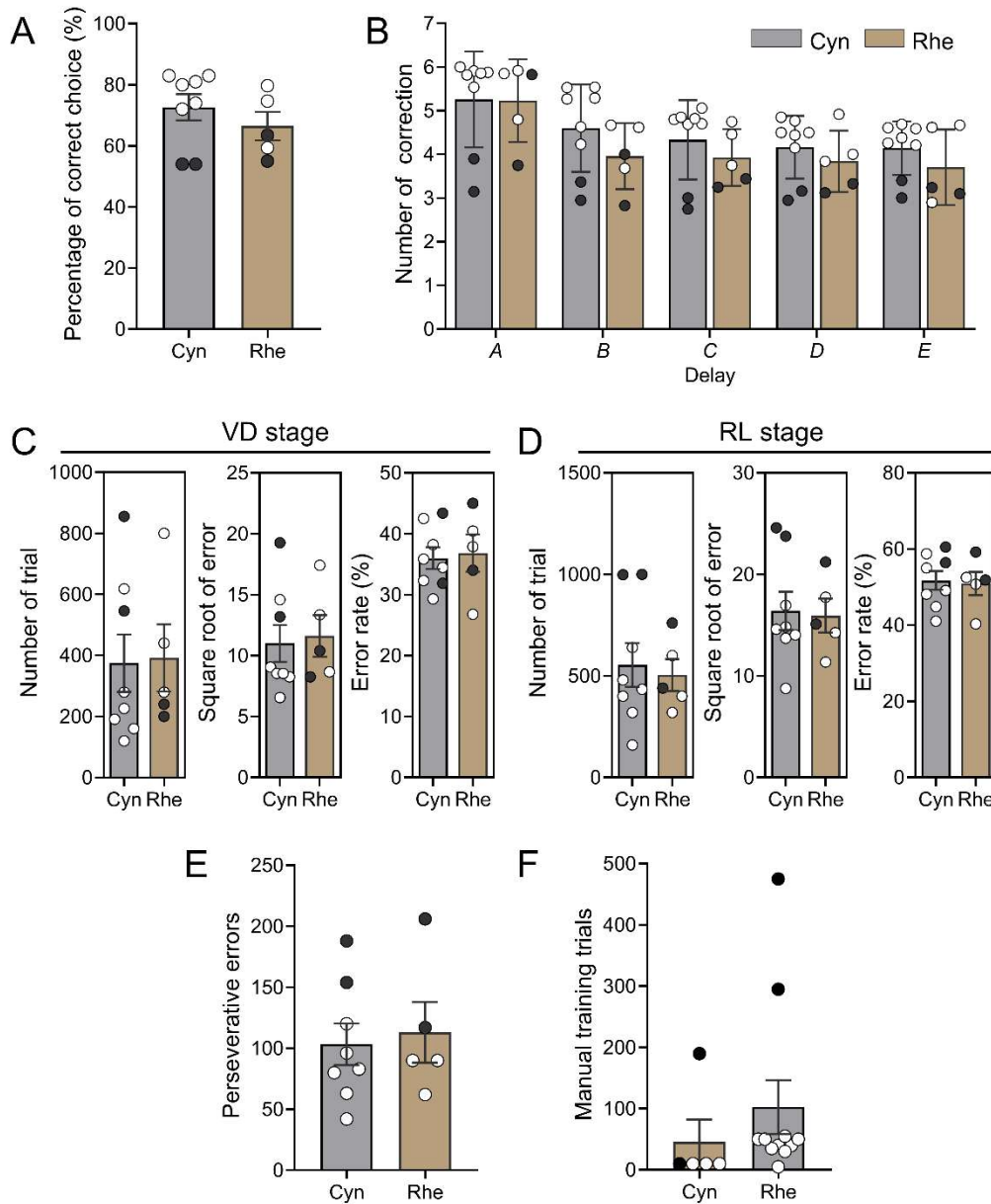


Fig. S3. Comparisons of cognitive performance between cynomolgus (Cyn) and rhesus (Rhe) monkeys.

(A) Percentage of correct choice at $B=2s$ in SDR task. (B) Number of corrections across five delays. (C-D) Number of trials to criteria, square of errors to criteria and error rate in VD stage (C) and RL stage (D) during the VD/RL task. (E) Perseverative errors after reversal during reversal learning task. (F) Manual training trials to reach criterion in operant learning task. Filled black circles represent KO monkeys, whereas open circles represent WT individuals. Data were presented as mean $\pm$ S.E.M. Mann-Whitney U test for (A) and (F); Student's t test for (E); Linear mixed-effects models for (B-D).

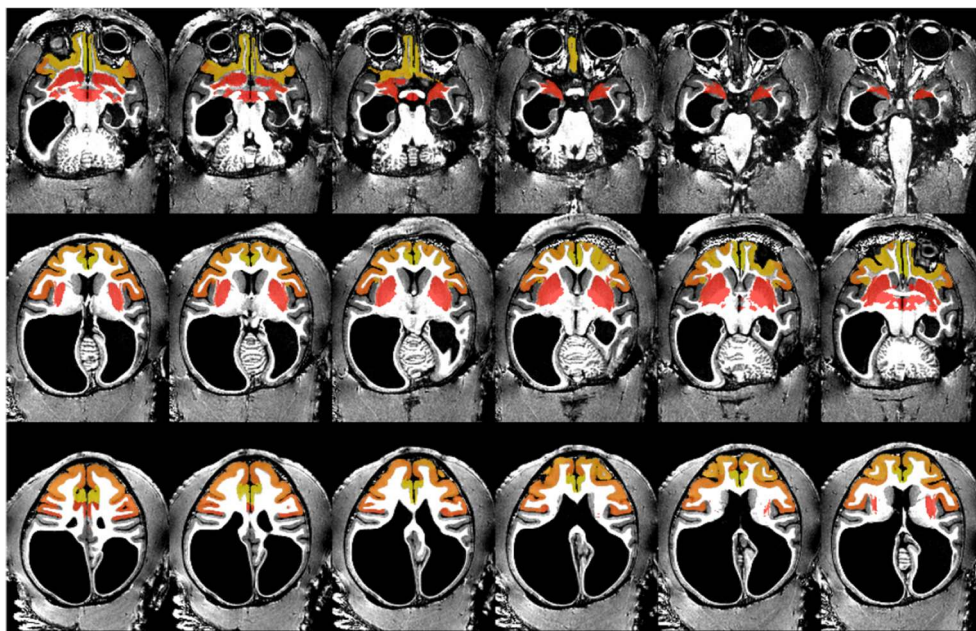


Fig S4. MRI/fMRI cortical and subcortical analysis mask.

Representative examples of cortical and subcortical masks used for MRI/fMRI analysis, overlaid on the T1-weighted images (monkey T87) showing the most pronounced ventricular abnormality. The masks illustrate the cortical and subcortical regions included in the quantitative analysis. Colors on the atlas denote distinct brain regions.

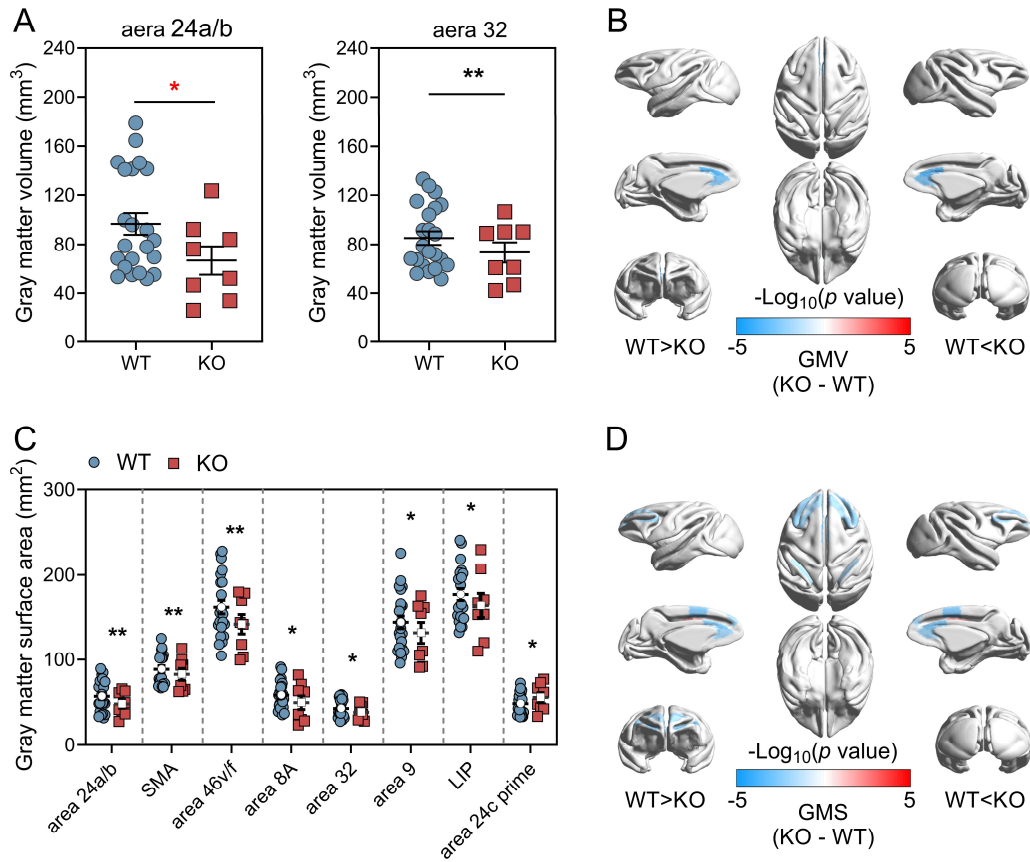


Fig. S5. Changes in gray matter volume and surface area in the giant *ANK2* knockout (KO) monkeys.

(A) Gray matter volume of regions with differences between KO and WT monkeys.

(B) Visualization of the regions in (A) on a monkey brain template.

(C) Gray matter surface area of regions with differences between KO and WT monkeys.

(D) Visualization of the regions in (C) on a monkey template.

Regions surviving FDR correction and regions showing group differences at the uncorrected threshold are displayed.

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$, KO versus WT; generalized linear mixed models corrected by the false discovery rate (FDR) method.

Red asterisks denoted false discovery rate (FDR)-corrected p values; black asterisks denoted uncorrected p values.

Data were presented as mean $\pm$ S.E.M.

This figure is related to Fig. 4. KO group, $n = 4$ (including 2 rhesus and 2 cynomolgus monkeys); WT group, $n = 11$ (including 5 rhesus and 6 cynomolgus monkeys).

The brain regions were defined in Table S3.

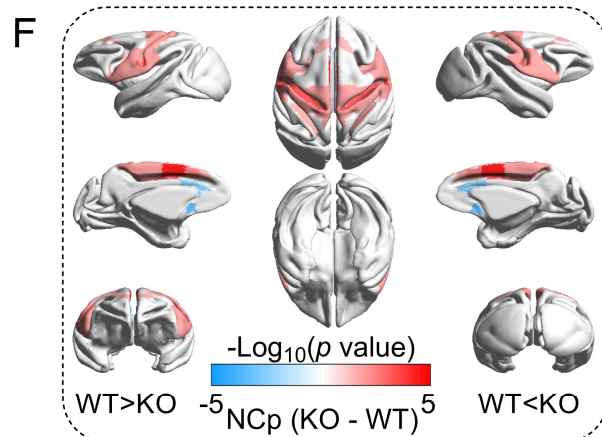
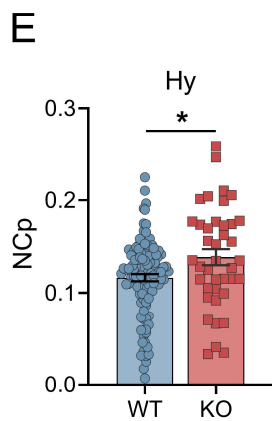
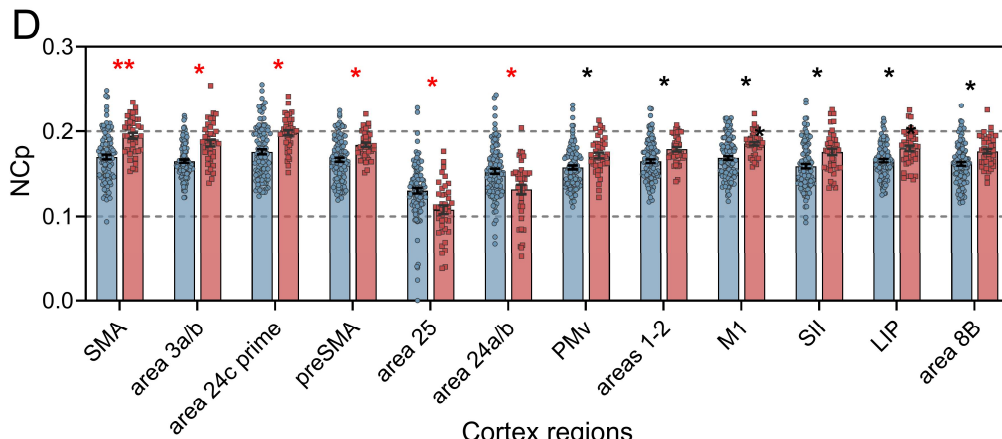
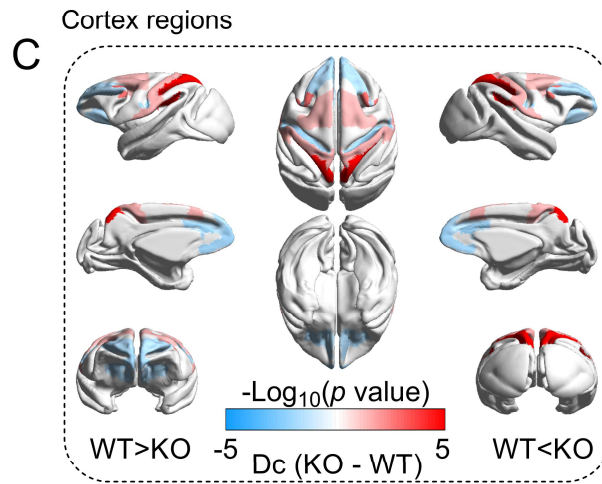
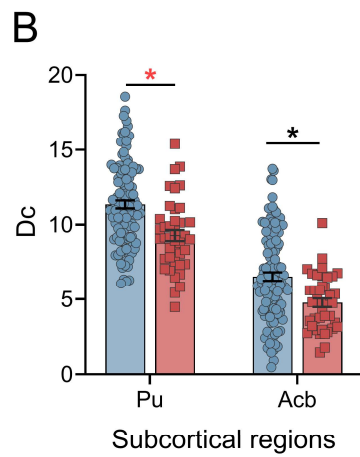
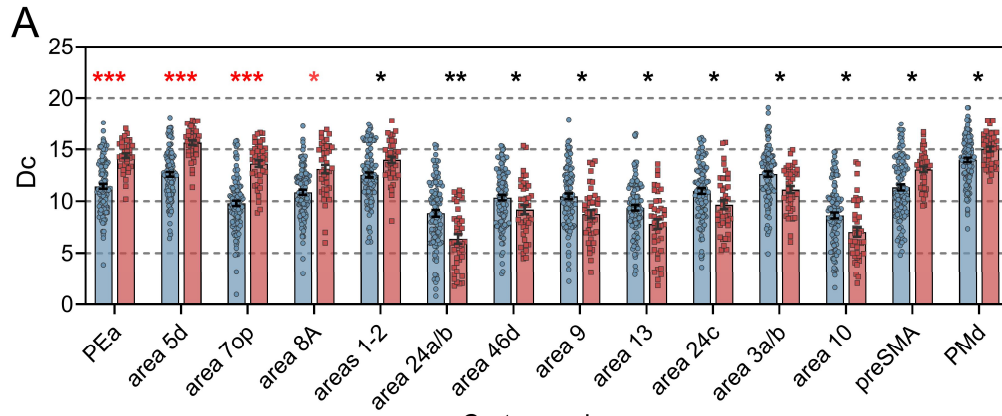


Fig. S6. Changes in degree centrality (Dc) and nodal clustering coefficient (NCp) in the giant *ANK2* knockout (KO) monkeys.

(A) Cortical regions with differences in Dc between KO and WT monkeys. (B) Subcortical regions with differences in Dc. (C) Visualization of the regions in (A) on a monkey brain template. (D) Cortical regions with differences in NCp. (E) Enhanced NCp of Hy from subcortical structure. (F) Visualization of the regions in (D) on a monkey brain template. Regions that survived FDR correction are marked with red asterisks (FDR-corrected p values), whereas regions showing group differences only at the uncorrected threshold are marked by black asterisks (uncorrected p values). * $p < 0.05$ **, $p < 0.01$ ***, $p < 0.001$, KO versus WT; generalized linear mixed models corrected by the false discovery rate (FDR) method. Data were presented as mean $\pm$ S.E.M. This figure is related to Fig. 4. KO group, $n = 4$ (including 2 rhesus and 2 cynomolgus monkeys); WT group, $n = 11$ (including 5 rhesus and 6 cynomolgus monkeys). The brain regions were defined in Table S3.

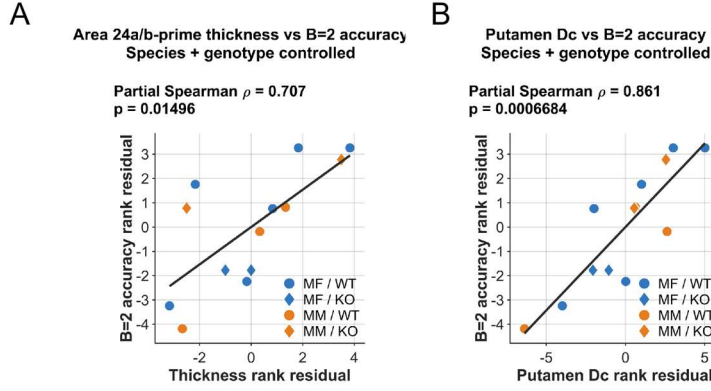


Fig. S7. Associations between regional neuroimaging measures and working memory performance.

(A-B) Partial Spearman rank correlations of working memory accuracy at $B = 2$ s with cortical thickness in area 24a/b prime (A) and putamen degree centrality (Dc) (B), controlling for species and genotype. Each point represents an individual monkey with available paired behavioral and neuroimaging data ($n = 13$). MF: *Macaca fascicularis*; MM: *Macaca mulatta*; KO, knockout; WT, wild-type.

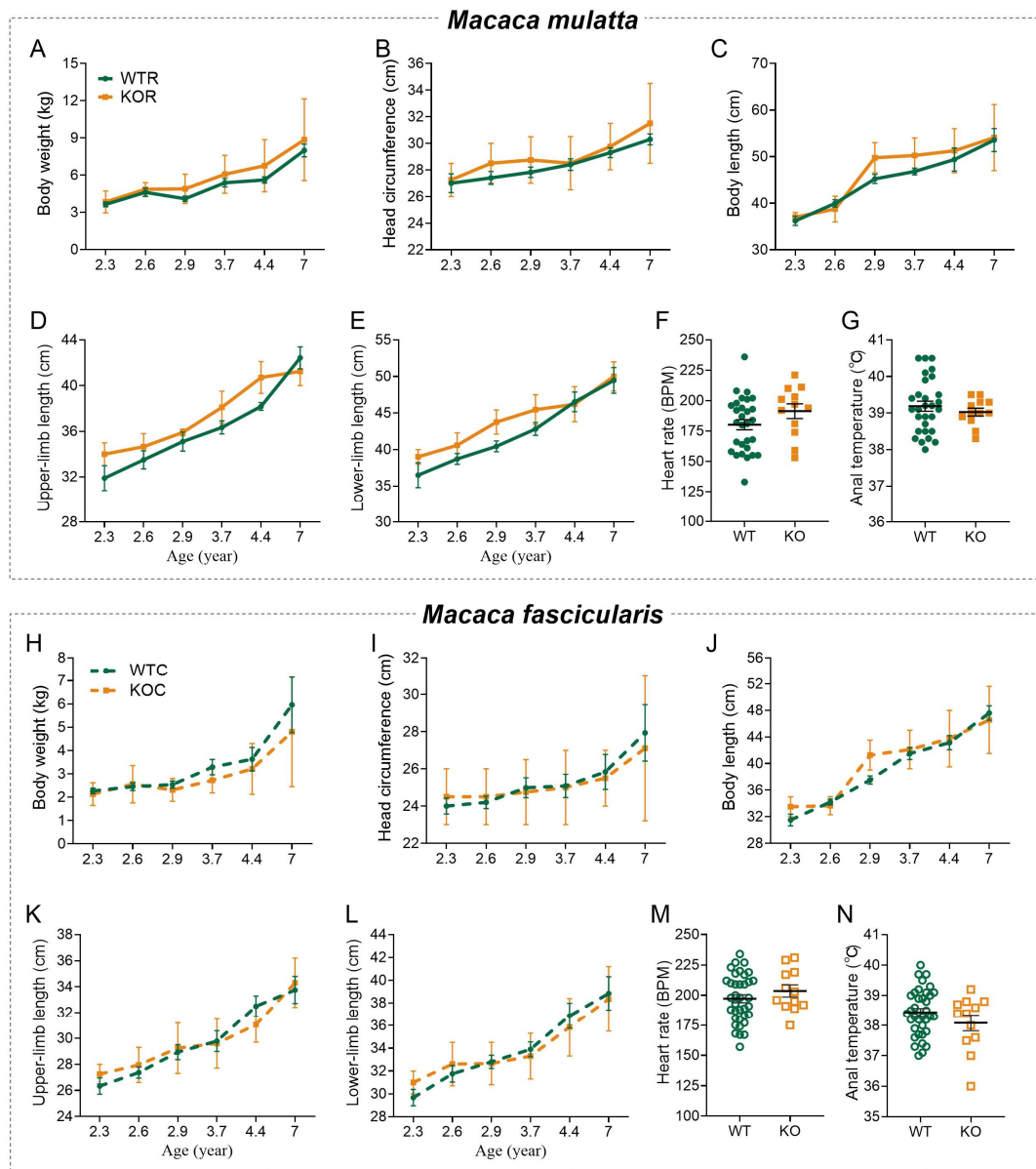


Fig. S8. No significant differences in developmental trajectory between the giant *ANK2* knockout (KO) and wild-type (WT) monkeys.

(A-E) Change of body weight (A), head circumference (B), body length (C), upper-limb length (D), and lower-limb length (E) across age in the KO and WT rhesus monkeys. (F-G) Heart rate (F) and (G) rectal temperatures in the KO and WT rhesus monkeys. (H-L) Change of body weight (H), head circumference (I), body length (J), upper-limb length (K), and lower-limb length (L) across age in the KO and WT cynomolgus monkeys. (M-N) Heart rate (M) and rectal temperature (N) in the KO and WT cynomolgus monkeys. Data were presented as mean $\pm$ S.E.M. Each data point

denotes a single measure for an individual, total six measures. (A-G): KO rhesus monkey (KOR) group, $n=2$; WT rhesus monkey (WTR) group, $n=5$; (H-N): KO cynomolgus monkey (KOC) group, $n = 2$; WT cynomolgus monkey (WTC) group, $n = 6$. Statistical analyses were performed by Student's t test or Mann-Whitney U test.

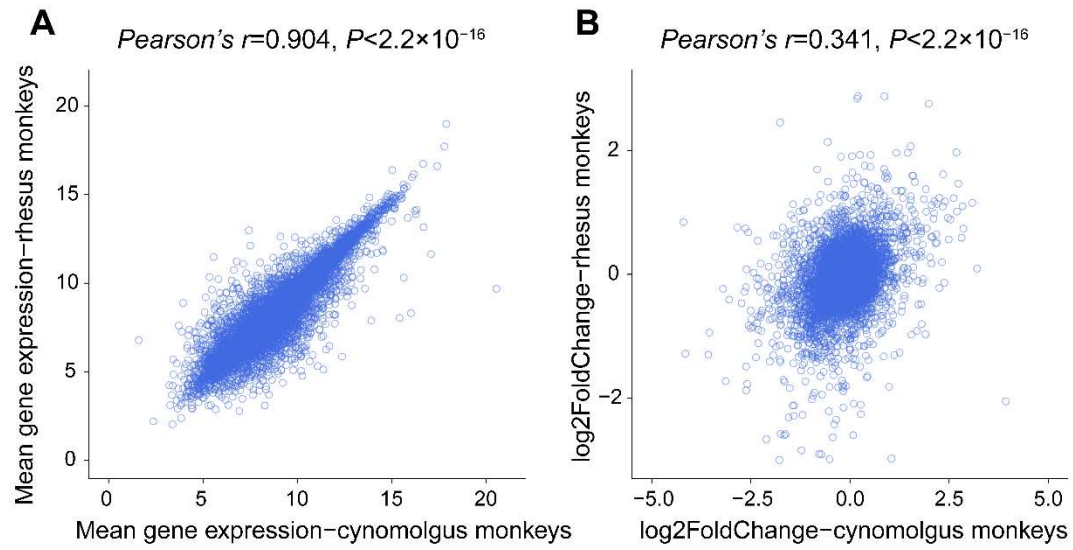


Fig. S10. Expression (A) and differential expression (B) of genes in peripheral blood between cynomolgus monkeys and rhesus monkeys. Mean gene expression (log2 normalized) and differential expression (represented by log2FoldChange) of 9182 human orthologous genes in two monkey species were used for analyses. Correlation coefficients and p values were calculated using Pearson's correlation.

Table S1 Characteristics and comparison of 46 kinematics in rhesus monkeys (*Macaca mulatta*) between the giant *ANK2* knockout (KO) and wild-type (WT)

Parameter	WT (<i>n</i> = 5)	KO (<i>n</i> = 2)	<i>t</i>	Corrected <i>p</i> value
Movement speed (mm/s)				
Nose	301.02 ± 63.63	756.64 ± 30.57	4.353	0.005 **
Left eye	280.96 ± 63.28	756.04 ± 34.67	4.544	0.005 **
Right eye	278.73 ± 63.74	741.6 ± 30.15	4.417	0.005 **
Head	263.81 ± 62.33	717.13 ± 38.69	4.378	0.005 **
Neck	237.6 ± 56.34	635.52 ± 20.91	4.320	0.005 **
Left shoulder	260.48 ± 50.99	572.41 ± 21.72	3.730	0.010 **
Right shoulder	261.23 ± 51.92	591.16 ± 17.62	3.892	0.009 **
Left elbow	340.82 ± 45.82	586.41 ± 22.44	3.257	0.014 *
Right elbow	338.8 ± 45.73	571.18 ± 20.39	3.096	0.016 *
Left wrist	461.34 ± 55.8	769.39 ± 54.01	3.213	0.014 *
Right wrist	456.39 ± 50.97	726.56 ± 24.51	3.222	0.014 *
Left limb	234.61 ± 30.86	395.71 ± 35.48	2.971	0.017 *
Right limb	240.97 ± 31.99	400.35 ± 55.47	2.598	0.027 *
Left knee	260.78 ± 30.97	384.84 ± 18.89	2.413	0.037 *
Right knee	269.05 ± 32.45	379.59 ± 23.51	2.033	0.068
Left ankle	268.13 ± 36.66	401.49 ± 14.09	2.223	0.049 *
Right ankle	273.5 ± 31.88	426.27 ± 31.95	2.777	0.021 *
Back	222.53 ± 32.87	381.95 ± 17.84	2.936	0.017 *
Tail root	232.05 ± 30.57	424.8 ± 73.64	2.922	0.017 *
Middle tail	265.08 ± 31.83	576.6 ± 140.74	3.181	0.014 *
Tail tip	368.24 ± 40.42	784.25 ± 207.83	2.986	0.017 *
Movement intensity (AU)				
Nose	11.88 ± 2.59	31.08 ± 2.21	4.377	0.005 *
Left eye	11.08 ± 2.58	30.84 ± 2.1	4.539	0.005 *
Right eye	11 ± 2.59	30.4 ± 2.16	4.427	0.005 *
Head	10.4 ± 2.54	29.15 ± 2.35	4.311	0.005 **
Neck	9.57 ± 2.29	25.62 ± 1.4	4.226	0.005 **
Left shoulder	10.21 ± 2.05	22.92 ± 1.37	3.714	0.010 **
Right shoulder	10.25 ± 2.1	23.47 ± 1.1	3.826	0.009 **
Left elbow	13.34 ± 1.77	23.83 ± 1.19	3.542	0.012 *
Right elbow	13.25 ± 1.78	23 ± 1.07	3.304	0.014 *
Left wrist	19.24 ± 2.28	31.87 ± 2.1	3.244	0.014 *
Right wrist	19.08 ± 2.03	30.27 ± 1.44	3.294	0.014 *
Left limb	9.34 ± 1.15	15.59 ± 1.57	3.004	0.017 *
Right limb	9.63 ± 1.2	15.81 ± 2.17	2.663	0.026 *
Left knee	9.92 ± 1.08	14.97 ± 0.76	2.777	0.021 *
Right knee	10.27 ± 1.13	14.68 ± 0.84	2.329	0.042 *
Left ankle	10.38 ± 1.38	15.97 ± 0.42	2.488	0.033 *
Right ankle	10.56 ± 1.2	16.67 ± 1.07	2.982	0.017 *

Back	8.78 ± 1.17	14.51 ± 0.76	2.933	0.017 *
Tail root	9.37 ± 1.11	17.66 ± 3.19	3.180	0.014 *
Middle tail	10.58 ± 1.14	24.19 ± 6.16	3.327	0.014 *
Tail tip	14.54 ± 1.47	33.29 ± 9.13	3.178	0.014 *
Distance per frame (mm)	5.6 ± 1.05	11.43 ± 0.44	3.387	0.014 *
Body length (mm)	421.39 ± 13.04	443.72 ± 21.33	0.907	0.391
Body height (mm)	198.28 ± 12.8	255.76 ± 12.59	2.609	0.027 *
Body angle (°)	90.34 ± 0.05	90.36 ± 0.05	0.248	0.808

Note: Data were presented as mean ± S.E. M. KO, giant *ANK2* knockout rhesus monkey; WT, wild-type rhesus monkey; AU, arbitrary units. *, corrected $p < 0.05$, **, corrected $p < 0.01$; multiple t tests with false discovery rate (FDR) correction.

Table S2 Description of the kinematic parameters and locomotive behaviors in 3D-motion analysis

Assessed parameters	Variables	Variable types	Definitions
Kinematic parameters	Movement speed	Velocity	The planar (x and y-plane) movement velocity per frame for 21 body key points of the monkey.
	Movement intensity	Velocity	The 3D movement velocity of 21 body key points, calculated as the 3D displacement per frame.
	Movement distance	Distance	The cumulative sum of planar (x and y-plane) displacement per frame.
	Body length	Length	The linear distance between nose and tail root, calculated as the Euclidean distance between the point of nose and root tail.
	Body height	Height	The average elevation of the monkey, computed by subtracting the mean z-coordinate of two fore wrists and two hind ankles from the z-coordinate of the back.
	Body angle	Angle	The angular configuration formed by neck, back, and tail root, derived from the angle between these three points.
Apparent displacement (AD) behaviors	Climb	Proportion of duration	Monkey ascends or moves horizontally with discernible velocity
	Fall	Proportion of duration	Monkey moves downward from the elevated position with discernible velocity
	Turn left	Proportion of duration	Monkey executes a leftward rotation in the lower cage region with rapid movement
	Turn right	Proportion of duration	Monkey executes rightward rotation in the lower cage region with rapid movement
	Walk	Proportion of duration	Monkey ambulates linearly in the lower cage region with rapid movement
	Jump	Proportion of duration	Monkey propels upward with significant height change and instantaneous high velocity
Non-apparent displacement (NAD) behaviors	Crouch	Proportion of duration	Monkey maintains a stationary curled posture in the lower cage region
	Stand up	Proportion of duration	Monkey elevates its body with at least one hind limb contacting the ground at low velocity
	Sit	Proportion of duration	Monkey maintains a flexed hindlimb posture in the lower cage region at low velocity
	Look left	Proportion of duration	Monkey orients head leftward ($>25^\circ$ deviation) in the lower cage region at low velocity
	Look right	Proportion of duration	Monkey orients head rightward ($>25^\circ$ deviation) in the lower cage region at low velocity
	Head rise	Proportion of duration	Monkey elevates its head in the lower cage region at low velocity
	Head lower	Proportion of duration	Monkey depresses its head in the lower cage region at low velocity
	Hang	Proportion of duration	Monkey clings to cage walls or ceiling in an elevated position with minimal movement velocity

Table S3 The abbreviations and region names of brain areas for neuroimaging analyses

Abbreviation	Region name	Abbreviation	Region name
Acb	accumbens	area 7op	parietal operculum
AIP	anterior intraparietal area	area 8A	periarculate area 8A (Frontal Eye Fields)
Amy	amygdala	area 8B	area 8B
area 10	frontal pole	area 9	area 9
area 11	area 11	areas 1-2	areas 1-2
area 12m/o	medial and orbital area 12	G	gustatory cortex
area 12r/l	rostral and lateral area 12	Hy	hypothalamus
area 13	area 13	Iam/Iapm	medial agranular insular region
area 14	gyrus rectus	lat Ia	lateral agranular insular region
area 23	area 23	LIP	lateral intraparietal area
area 24a/b	areas 24a and 24b	LVPal	lateral and ventral pallium
area 24a/b prime	areas 24a' and 24b'	M1	primary motor cortex
area 24c	area 24c	OLF	primary olfactory cortex
area 24c prime	area 24c'	Pd	pallidum
area 25	subgenual cortex	PEa	area PEa
area 31	area 31	PMd	dorsal premotor cortex
area 32	area 32	PMv	ventral premotor cortex
area 3a/b	areas 3a and 3b	PrCO	precentral operular area
area 44	area 44	preSMA	presupplementary motor area
area 45	area 45	Pu	putamen
area 46d	dorsal area 46	SII	secondary somatosensory cortex
area 46v/f	ventral and fundus portion of area 46	SMA	supplementary motor area
area 5d	dorsal area 5		

Table S4 Comparison of the structural and functional MRI parameters in cortical regions between the giant *ANK2* knockout (KO) and wild-type (WT) monkeys

Parameter	Region	WT (<i>n</i> = 11)	KO (<i>n</i> = 4)	<i>p</i> value	Corrected <i>p</i> value
Myelination	area 32	0.104 ± 0.002	0.103 ± 0.008	0.775	0.843
	area 25	0.106 ± 0.002	0.111 ± 0.006	0.215	0.448
	area 24a/b	0.104 ± 0.002	0.107 ± 0.010	0.484	0.751
	area 24c	0.104 ± 0.002	0.104 ± 0.010	0.963	0.985
	area 24a/b prime	0.123 ± 0.002	0.130 ± 0.009	0.241	0.472
	area 24c prime	0.126 ± 0.002	0.129 ± 0.009	0.757	0.843
	area 10	0.107 ± 0.002	0.109 ± 0.007	0.527	0.774
	area 11	0.112 ± 0.002	0.119 ± 0.007	0.136	0.418
	area 13	0.117 ± 0.002	0.123 ± 0.006	0.208	0.448
	area 12m/o	0.117 ± 0.002	0.125 ± 0.006	0.150	0.418
	Iam/Iapm	0.111 ± 0.003	0.123 ± 0.004	0.040*	0.301
	lat Ia	0.108 ± 0.002	0.114 ± 0.006	0.186	0.440
	OLF	0.116 ± 0.002	0.119 ± 0.006	0.550	0.774
	G	0.126 ± 0.002	0.124 ± 0.007	0.658	0.834
	PrCO	0.113 ± 0.002	0.115 ± 0.007	0.699	0.834
	area 8A	0.141 ± 0.003	0.148 ± 0.010	0.365	0.619
	area 8B	0.127 ± 0.002	0.131 ± 0.010	0.574	0.782
	area 9	0.109 ± 0.002	0.114 ± 0.011	0.407	0.654
	area 46d	0.119 ± 0.002	0.124 ± 0.009	0.313	0.563
	area 46v/f	0.119 ± 0.002	0.122 ± 0.010	0.547	0.774
	area 12r/l	0.117 ± 0.002	0.123 ± 0.008	0.267	0.501
	area 45	0.136 ± 0.002	0.139 ± 0.009	0.616	0.815
	area 44	0.127 ± 0.002	0.126 ± 0.009	0.915	0.957
	M1	0.169 ± 0.003	0.184 ± 0.008	0.036*	0.301
	PMd	0.150 ± 0.003	0.162 ± 0.009	0.098	0.418
	PMv	0.139 ± 0.002	0.141 ± 0.009	0.704	0.834
	preSMA	0.116 ± 0.002	0.118 ± 0.011	0.724	0.836
	SMA	0.134 ± 0.002	0.143 ± 0.009	0.166	0.418
	area 3a/b	0.163 ± 0.003	0.173 ± 0.008	0.167	0.418
	areas 1-2	0.155 ± 0.003	0.164 ± 0.009	0.219	0.448
	SII	0.136 ± 0.003	0.138 ± 0.008	0.787	0.843
	area 5d	0.141 ± 0.003	0.151 ± 0.008	0.161	0.418
	PEa	0.151 ± 0.003	0.163 ± 0.008	0.114	0.418
	AIP	0.150 ± 0.003	0.161 ± 0.009	0.162	0.418
	LIP	0.145 ± 0.003	0.156 ± 0.007	0.119	0.418
	area 7op	0.149 ± 0.004	0.152 ± 0.008	0.667	0.834
	area 31	0.132 ± 0.003	0.144 ± 0.007	0.057	0.365

	area 23	0.129 ± 0.003	0.134 ± 0.007	0.371	0.619
Gray matter volume	area 32	85.261 ± 5.466	73.469 ± 8.352	0.006**	0.090
	area 25	45.886 ± 2.888	42.094 ± 5.130	0.171	0.649
	area 24a/b	96.653 ± 8.782	66.781 ± 11.665	0.001***	0.024*
	area 24c	176.290 ± 10.511	182.922 ± 20.026	0.788	0.959
	area 24a/b prime	93.455 ± 6.401	83.516 ± 11.609	0.099	0.555
	area 24c prime	116.369 ± 6.005	120.063 ± 10.506	0.789	0.959
	area 10	187.903 ± 6.809	188.859 ± 27.410	0.924	1.000
	area 11	111.358 ± 7.548	107.188 ± 16.981	0.510	0.919
	area 13	367.256 ± 26.427	345.625 ± 40.894	0.232	0.653
	area 12m/o	145.864 ± 9.449	135.234 ± 11.315	0.154	0.649
	Iam/Iapm	24.080 ± 1.858	25.781 ± 3.229	0.625	0.959
	lat Ia	154.858 ± 9.848	163.969 ± 21.750	0.645	0.959
	OLF	49.693 ± 2.530	51.172 ± 5.086	0.848	0.978
	G	44.369 ± 2.824	47.453 ± 5.473	0.510	0.919
	PrCO	78.898 ± 4.542	74.219 ± 9.696	0.386	0.875
	area 8A	76.830 ± 4.764	67.688 ± 11.659	0.061	0.459
	area 8B	203.665 ± 9.499	211.016 ± 24.101	0.753	0.959
	area 9	240.983 ± 14.147	227.406 ± 24.689	0.208	0.649
	area 46d	209.489 ± 11.645	205.391 ± 28.589	0.589	0.959
	area 46v/f	268.483 ± 14.847	242.344 ± 25.202	0.076	0.489
	area 12r/l	68.665 ± 7.496	59.953 ± 16.609	0.408	0.875
	area 45	144.261 ± 8.012	134.281 ± 13.101	0.179	0.649
	area 44	44.131 ± 2.237	43.609 ± 6.685	0.727	0.959
	Ml	693.091 ± 35.158	665.438 ± 59.595	0.281	0.743
	PMd	696.773 ± 40.408	766.594 ± 88.381	0.216	0.649
	PMv	586.767 ± 27.591	595.156 ± 74.783	0.955	1.000
	preSMA	77.114 ± 4.505	75.250 ± 9.696	0.480	0.919
	SMA	154.989 ± 8.686	142.906 ± 15.368	0.997	1.000
	area 3a/b	240.068 ± 12.301	240.375 ± 23.068	0.828	0.978
	areas 1-2	623.364 ± 30.926	601.781 ± 53.214	0.330	0.825
	SII	264.710 ± 12.337	304.172 ± 42.663	0.113	0.563
	area 5d	205.438 ± 10.945	196.906 ± 27.705	0.481	0.919
	PEa	184.818 ± 8.676	172.938 ± 15.310	0.204	0.649
	AIP	46.403 ± 2.268	45.906 ± 4.902	0.731	0.959
	LIP	252.426 ± 12.701	228.719 ± 25.483	0.054	0.459
	area 7op	170.403 ± 9.010	169.844 ± 18.770	0.689	0.959
	area 31	38.244 ± 3.260	41.656 ± 4.828	0.536	0.928
	area 23	293.006 ± 16.834	299.531 ± 28.876	0.972	1.000
Thickness	area 32	2.012 ± 0.033	1.867 ± 0.073	0.019*	0.147
	area 25	1.864 ± 0.027	1.706 ± 0.046	0.003**	0.033*
	area 24a/b	2.011 ± 0.053	1.521 ± 0.114	<0.001***	<0.001***
	area 24c	1.982 ± 0.028	2.218 ± 0.064	0.001***	0.007**

	area 24a/b prime	1.620 ± 0.041	1.362 ± 0.105	<0.001***	0.007**
	area 24c prime	1.786 ± 0.019	1.760 ± 0.027	0.481	0.794
	area 10	1.652 ± 0.028	1.605 ± 0.090	0.314	0.757
	area 11	1.525 ± 0.039	1.553 ± 0.111	0.822	0.958
	area 13	1.946 ± 0.029	1.904 ± 0.071	0.408	0.757
	area 12m/o	1.960 ± 0.049	1.975 ± 0.071	0.949	1.000
	Iam/Iapm	1.669 ± 0.066	1.748 ± 0.091	0.536	0.809
	lat Ia	2.124 ± 0.057	2.120 ± 0.095	0.816	0.958
	OLF	1.854 ± 0.052	1.912 ± 0.090	0.589	0.851
	G	1.666 ± 0.031	1.738 ± 0.042	0.217	0.705
	PrCO	2.313 ± 0.050	2.353 ± 0.045	0.685	0.954
	area 8A	1.900 ± 0.021	1.894 ± 0.050	0.836	0.958
	area 8B	2.091 ± 0.023	2.128 ± 0.030	0.391	0.757
	area 9	2.132 ± 0.032	2.212 ± 0.040	0.180	0.666
	area 46d	1.735 ± 0.021	1.781 ± 0.031	0.259	0.757
	area 46v/f	1.542 ± 0.017	1.592 ± 0.041	0.188	0.666
	area 12r/l	1.897 ± 0.034	1.783 ± 0.136	0.161	0.666
	area 45	1.958 ± 0.020	1.975 ± 0.044	0.725	0.958
	area 44	1.731 ± 0.022	1.784 ± 0.078	0.383	0.757
	M1	2.185 ± 0.021	2.099 ± 0.056	0.061	0.397
	PMd	2.394 ± 0.021	2.410 ± 0.044	0.751	0.958
	PMv	2.163 ± 0.020	2.193 ± 0.056	0.540	0.809
	preSMA	2.204 ± 0.029	2.274 ± 0.073	0.285	0.757
	SMA	2.251 ± 0.032	2.213 ± 0.092	0.489	0.794
	area 3a/b	1.332 ± 0.018	1.332 ± 0.025	0.989	1.000
	areas 1-2	1.725 ± 0.020	1.694 ± 0.039	0.349	0.757
	SII	1.861 ± 0.021	1.866 ± 0.059	0.968	1.000
	area 5d	1.855 ± 0.030	1.859 ± 0.072	0.981	1.000
	PEa	1.677 ± 0.027	1.633 ± 0.046	0.378	0.757
	AIP	1.563 ± 0.022	1.491 ± 0.053	0.146	0.666
	LIP	1.680 ± 0.021	1.649 ± 0.044	0.374	0.757
	area 7op	1.649 ± 0.021	1.591 ± 0.032	0.075	0.419
	area 31	1.881 ± 0.046	1.910 ± 0.118	0.819	0.958
	area 23	1.648 ± 0.018	1.677 ± 0.046	0.488	0.794
Surface area	area 32	42.625 ± 2.347	38.406 ± 3.139	0.027*	0.198
	area 25	27.341 ± 1.689	28.375 ± 3.298	0.825	0.919
	area 24a/b	57.000 ± 3.997	48.063 ± 4.919	0.004**	0.100
	area 24c	69.421 ± 4.587	64.906 ± 8.223	0.090	0.320
	area 24a/b prime	68.330 ± 3.404	67.750 ± 6.039	0.674	0.874
	area 24c prime	48.386 ± 2.552	55.625 ± 5.643	0.036*	0.200
	area 10	152.636 ± 3.731	150.906 ± 15.551	0.745	0.888
	area 11	82.932 ± 4.028	75.719 ± 7.259	0.068	0.296
	area 13	193.784 ± 12.057	186.656 ± 16.673	0.269	0.548

	area 12m/o	88.784 ± 4.556	82.531 ± 6.249	0.197	0.521
	Iam/Iapm	14.261 ± 0.681	15.313 ± 1.733	0.372	0.580
	lat Ia	71.523 ± 3.044	76.969 ± 8.478	0.306	0.548
	OLF	29.352 ± 1.024	29.219 ± 2.808	0.816	0.919
	G	15.034 ± 0.968	15.469 ± 2.129	0.951	0.976
	PrCO	43.205 ± 1.731	40.219 ± 4.901	0.266	0.548
	area 8A	58.261 ± 3.394	49.469 ± 7.944	0.017*	0.169
	area 8B	102.557 ± 4.457	102.688 ± 11.204	0.752	0.888
	area 9	144.080 ± 7.545	131.531 ± 12.492	0.031*	0.198
	area 46d	151.705 ± 7.899	145.125 ± 18.889	0.301	0.548
	area 46v/f	161.943 ± 7.906	141.781 ± 11.519	0.009**	0.120
	area 12r/l	50.625 ± 4.593	43.969 ± 9.874	0.280	0.548
	area 45	88.546 ± 4.394	82.313 ± 6.299	0.152	0.455
	area 44	14.773 ± 0.689	14.188 ± 1.912	0.474	0.684
	Ml	349.784 ± 14.900	346.188 ± 23.394	0.502	0.699
	PMd	327.682 ± 17.010	357.438 ± 35.902	0.200	0.521
	PMv	322.864 ± 12.532	320.281 ± 32.075	0.638	0.858
	preSMA	44.534 ± 2.387	41.094 ± 5.136	0.101	0.327
	SMA	88.534 ± 4.109	82.500 ± 7.000	0.005**	0.100
	area 3a/b	173.830 ± 8.953	174.844 ± 14.853	0.872	0.919
	areas 1-2	451.386 ± 18.514	438.781 ± 29.643	0.336	0.570
	SII	146.705 ± 4.872	164.094 ± 17.169	0.080	0.314
	area 5d	146.261 ± 6.855	137.125 ± 15.347	0.247	0.548
	PEa	120.364 ± 4.628	116.875 ± 6.549	0.395	0.592
	AIP	21.580 ± 1.199	23.781 ± 2.293	0.309	0.548
	LIP	176.830 ± 7.005	163.969 ± 14.237	0.045*	0.217
	area 7op	98.375 ± 4.675	100.938 ± 9.354	0.869	0.919
	area 3l	23.932 ± 1.951	27.844 ± 4.904	0.357	0.580
	area 23	168.761 ± 9.318	168.156 ± 14.484	0.695	0.874
Functional connectivity density (FCD)	area 32	0.082 ± 0.003	0.071 ± 0.003	0.229	0.479
	area 25	0.064 ± 0.002	0.054 ± 0.003	0.068	0.305
	area 24a/b	0.102 ± 0.004	0.072 ± 0.004	0.010**	0.088
	area 24c	0.125 ± 0.005	0.104 ± 0.005	0.142	0.400
	area 24a/b prime	0.108 ± 0.005	0.096 ± 0.005	0.366	0.514
	area 24c prime	0.125 ± 0.005	0.129 ± 0.006	0.857	0.857
	area 10	0.100 ± 0.005	0.078 ± 0.005	0.046*	0.259
	area 14	0.075 ± 0.003	0.061 ± 0.003	0.036*	0.234
	area 11	0.090 ± 0.004	0.079 ± 0.005	0.259	0.485
	area 13	0.105 ± 0.004	0.084 ± 0.005	0.108	0.348
	area 12m/o	0.093 ± 0.005	0.079 ± 0.004	0.245	0.479
	Iam/Iapm	0.072 ± 0.003	0.059 ± 0.003	0.064	0.305
	lat Ia	0.088 ± 0.003	0.074 ± 0.004	0.087	0.338
	OLF	0.066 ± 0.002	0.059 ± 0.003	0.165	0.438

	G	0.072 ± 0.003	0.063 ± 0.004	0.300	0.509
	PrCO	0.073 ± 0.003	0.067 ± 0.004	0.333	0.514
	area 8A	0.123 ± 0.005	0.152 ± 0.008	0.090	0.338
	area 8B	0.147 ± 0.006	0.161 ± 0.007	0.508	0.653
	area 9	0.120 ± 0.005	0.098 ± 0.005	0.128	0.385
	area 46d	0.119 ± 0.005	0.102 ± 0.005	0.194	0.460
	area 46v/f	0.132 ± 0.006	0.113 ± 0.006	0.320	0.514
	area 12r/l	0.094 ± 0.005	0.079 ± 0.003	0.189	0.460
	area 45	0.106 ± 0.004	0.110 ± 0.006	0.808	0.845
	area 44	0.088 ± 0.004	0.079 ± 0.005	0.355	0.514
	M1	0.170 ± 0.006	0.181 ± 0.007	0.603	0.733
	PMd	0.168 ± 0.007	0.187 ± 0.007	0.365	0.514
	PMv	0.123 ± 0.005	0.129 ± 0.006	0.703	0.809
	preSMA	0.131 ± 0.006	0.141 ± 0.006	0.692	0.809
	SMA	0.136 ± 0.007	0.145 ± 0.007	0.737	0.809
	area 3a/b	0.150 ± 0.006	0.129 ± 0.006	0.106	0.348
	areas 1-2	0.148 ± 0.006	0.168 ± 0.007	0.242	0.479
	SII	0.110 ± 0.004	0.120 ± 0.006	0.379	0.517
	area 5d	0.151 ± 0.005	0.201 ± 0.006	<0.001***	0.011*
	PEa	0.139 ± 0.005	0.182 ± 0.006	0.007**	0.085
	AIP	0.085 ± 0.003	0.083 ± 0.004	0.725	0.809
	LIP	0.166 ± 0.006	0.184 ± 0.006	0.305	0.509
	area 7op	0.113 ± 0.004	0.159 ± 0.006	<0.001***	0.011*
	area 3l	0.127 ± 0.005	0.137 ± 0.005	0.554	0.692
	area 23	0.158 ± 0.006	0.172 ± 0.005	0.464	0.615
Degree centrality (Dc)	area 32	6.917 ± 0.260	6.309 ± 0.371	0.442	0.560
	area 25	5.332 ± 0.234	4.541 ± 0.372	0.233	0.392
	area 24a/b	8.835 ± 0.310	6.377 ± 0.457	0.009**	0.069
	area 24c	10.977 ± 0.266	9.661 ± 0.434	0.044*	0.125
	area 24a/b prime	9.516 ± 0.339	8.725 ± 0.455	0.474	0.576
	area 24c prime	10.788 ± 0.246	11.476 ± 0.283	0.301	0.452
	area 10	8.627 ± 0.285	7.034 ± 0.450	0.018*	0.101
	area 14	6.311 ± 0.251	5.186 ± 0.356	0.095	0.224
	area 11	7.811 ± 0.263	7.088 ± 0.488	0.326	0.474
	area 13	9.362 ± 0.250	7.811 ± 0.477	0.035*	0.120
	area 12m/o	7.981 ± 0.283	7.283 ± 0.399	0.371	0.522
	Iam/Iapm	5.980 ± 0.267	5.096 ± 0.365	0.189	0.354
	lat Ia	7.686 ± 0.282	6.463 ± 0.366	0.123	0.251
	OLF	5.508 ± 0.289	5.066 ± 0.326	0.636	0.666
	G	5.997 ± 0.249	5.514 ± 0.383	0.396	0.540
	PrCO	6.004 ± 0.227	5.923 ± 0.331	0.881	0.901
	area 8A	10.872 ± 0.236	13.102 ± 0.404	0.002**	0.020*
	area 8B	12.748 ± 0.289	13.861 ± 0.307	0.235	0.392

	area 9	10.467 ± 0.273	8.771 ± 0.408	0.033*	0.120
	area 46d	10.340 ± 0.266	9.183 ± 0.439	0.035*	0.120
	area 46v/f	11.632 ± 0.310	10.461 ± 0.468	0.245	0.393
	area 12r/l	7.884 ± 0.304	7.060 ± 0.360	0.286	0.443
	area 45	9.299 ± 0.227	10.356 ± 0.417	0.089	0.222
	area 44	7.491 ± 0.251	6.994 ± 0.398	0.486	0.576
	M1	13.839 ± 0.211	14.324 ± 0.248	0.418	0.553
	PMd	13.975 ± 0.217	15.031 ± 0.240	0.045*	0.125
	PMv	10.811 ± 0.252	11.720 ± 0.424	0.230	0.392
	preSMA	11.330 ± 0.286	13.066 ± 0.285	0.035*	0.120
	SMA	11.619 ± 0.297	12.361 ± 0.361	0.448	0.560
	area 3a/b	12.616 ± 0.251	11.117 ± 0.337	0.016*	0.101
	areas 1-2	12.528 ± 0.246	13.973 ± 0.308	0.037*	0.120
	SII	9.506 ± 0.252	10.771 ± 0.426	0.052	0.137
	area 5d	12.595 ± 0.229	15.631 ± 0.233	<0.001***	<0.001***
	PEa	11.429 ± 0.244	14.377 ± 0.229	<0.001***	<0.001***
	AIP	7.201 ± 0.262	7.619 ± 0.427	0.626	0.666
	LIP	13.875 ± 0.227	14.848 ± 0.238	0.102	0.230
	area 7op	9.804 ± 0.258	13.618 ± 0.318	<0.001***	<0.001***
	area 3l	10.896 ± 0.250	11.884 ± 0.361	0.176	0.344
	area 23	13.203 ± 0.222	14.099 ± 0.223	0.109	0.233
Nodal clustering coefficient (NCp)	area 32	0.148 ± 0.003	0.147 ± 0.005	0.849	0.957
	area 25	0.130 ± 0.003	0.108 ± 0.005	0.001**	0.020*
	area 24a/b	0.153 ± 0.003	0.132 ± 0.006	0.005**	0.034*
	area 24c	0.159 ± 0.003	0.162 ± 0.004	0.770	0.937
	area 24a/b prime	0.159 ± 0.003	0.173 ± 0.005	0.131	0.307
	area 24c prime	0.176 ± 0.003	0.198 ± 0.003	0.002**	0.022*
	area 10	0.154 ± 0.003	0.144 ± 0.005	0.144	0.308
	area 14	0.137 ± 0.003	0.128 ± 0.004	0.080	0.232
	area 11	0.154 ± 0.003	0.158 ± 0.006	0.726	0.907
	area 13	0.151 ± 0.003	0.150 ± 0.005	0.890	0.975
	area 12m/o	0.151 ± 0.003	0.150 ± 0.005	0.851	0.957
	Iam/Iapm	0.133 ± 0.003	0.132 ± 0.007	0.910	0.975
	lat Ia	0.139 ± 0.003	0.133 ± 0.005	0.420	0.590
	OLF	0.128 ± 0.003	0.116 ± 0.005	0.168	0.329
	G	0.149 ± 0.004	0.138 ± 0.006	0.230	0.423
	PrCO	0.142 ± 0.003	0.142 ± 0.006	0.943	0.986
	area 8A	0.170 ± 0.003	0.178 ± 0.004	0.412	0.590
	area 8B	0.162 ± 0.002	0.176 ± 0.003	0.027*	0.095
	area 9	0.162 ± 0.003	0.171 ± 0.004	0.284	0.473
	area 46d	0.164 ± 0.003	0.176 ± 0.005	0.243	0.423
	area 46v/f	0.157 ± 0.002	0.165 ± 0.005	0.300	0.483
	area 12r/l	0.152 ± 0.003	0.157 ± 0.004	0.546	0.702

	area 45	0.158 ± 0.003	0.164 ± 0.004	0.503	0.685
	area 44	0.156 ± 0.003	0.156 ± 0.005	0.969	0.991
	M1	0.168 ± 0.002	0.185 ± 0.002	0.018*	0.072
	PMd	0.168 ± 0.002	0.181 ± 0.003	0.087	0.232
	PMv	0.157 ± 0.002	0.171 ± 0.003	0.014*	0.072
	preSMA	0.166 ± 0.002	0.184 ± 0.003	0.003**	0.029*
	SMA	0.170 ± 0.003	0.195 ± 0.003	<0.001***	0.002**
	area 3a/b	0.165 ± 0.002	0.186 ± 0.004	<0.001***	0.013*
	areas 1-2	0.165 ± 0.002	0.179 ± 0.003	0.017*	0.072
	SII	0.159 ± 0.003	0.175 ± 0.004	0.013*	0.072
	area 5d	0.173 ± 0.003	0.176 ± 0.002	0.803	0.951
	PEa	0.175 ± 0.003	0.184 ± 0.003	0.353	0.530
	AIP	0.166 ± 0.003	0.183 ± 0.004	0.065	0.210
	LIP	0.165 ± 0.002	0.180 ± 0.003	0.023*	0.085
	area 7op	0.175 ± 0.003	0.184 ± 0.002	0.136	0.307
	area 3l	0.175 ± 0.003	0.188 ± 0.003	0.168	0.329
	area 23	0.171 ± 0.003	0.183 ± 0.003	0.084	0.232

Note: Data were presented as mean ± S.E.M. KO, $n = 4$ giant *ANK2* knockout monkeys, including 2 rhesus and 2 cynomolgus monkeys; WT, $n = 11$ wild-type monkeys, including 5 rhesus and 6 cynomolgus monkeys; FCD, functional connectivity density; Dc, degree centrality; NCp, nodal clustering coefficient.

*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Table S5 Comparison of the structural and functional MRI parameters in subcortical regions between the giant *ANK2* knockout (KO) and wild-type (WT) monkeys

Parameter	Region	WT (<i>n</i> = 11)	KO (<i>n</i> = 4)	<i>p</i> value	Corrected <i>p</i> value
Myelination	LVPal	0.185 ± 0.003	0.197 ± 0.010	0.108	0.418
	Amy	0.126 ± 0.003	0.137 ± 0.008	0.093	0.418
	Pu	0.199 ± 0.003	0.221 ± 0.010	0.008***	0.117
	Acb	0.139 ± 0.002	0.150 ± 0.006	0.036*	0.301
	Pd	0.361 ± 0.007	0.419 ± 0.017	0.001***	0.019*
	Hy	0.219 ± 0.004	0.257 ± 0.009	<0.001***	0.004**
Volume	LVPal	271.131 ± 13.476	274.688 ± 23.973	0.922	1.000
	Amy	262.182 ± 9.485	269.016 ± 15.517	0.753	0.959
	Pu	767.722 ± 31.513	757.625 ± 50.109	0.395	0.875
	Acb	87.216 ± 3.620	87.063 ± 9.263	0.759	0.959
	Pd	218.046 ± 9.303	191.125 ± 9.768	0.001***	0.030*
	Hy	159.602 ± 5.907	151.000 ± 7.260	0.055	0.459
Functional connectivity density (FCD)	LVPal	0.102 ± 0.004	0.091 ± 0.005	0.283	0.509
	Amy	0.066 ± 0.003	0.065 ± 0.004	0.827	0.846
	Pu	0.127 ± 0.005	0.099 ± 0.005	0.017*	0.126
	Acb	0.077 ± 0.003	0.057 ± 0.003	0.008**	0.085
	Pd	0.055 ± 0.002	0.049 ± 0.003	0.214	0.479
	Hy	0.054 ± 0.002	0.052 ± 0.003	0.791	0.845
Degree centrality (Dc)	LVPal	9.033 ± 0.273	8.607 ± 0.476	0.564	0.619
	Amy	5.465 ± 0.261	5.907 ± 0.431	0.523	0.603
	Pu	11.350 ± 0.268	9.277 ± 0.375	0.002**	0.019*
	Acb	6.525 ± 0.289	4.762 ± 0.292	0.024*	0.120
	Pd	4.377 ± 0.218	3.969 ± 0.329	0.546	0.614
	Hy	4.308 ± 0.239	4.352 ± 0.357	0.941	0.941
Nodal clustering coefficient (NCp)	LVPal	0.155 ± 0.003	0.148 ± 0.005	0.343	0.530
	Amy	0.138 ± 0.004	0.148 ± 0.006	0.245	0.423
	Pu	0.160 ± 0.003	0.161 ± 0.004	0.993	0.993
	Acb	0.136 ± 0.003	0.124 ± 0.005	0.103	0.258
	Pd	0.133 ± 0.004	0.128 ± 0.007	0.535	0.702
	Hy	0.116 ± 0.004	0.139 ± 0.009	0.016*	0.072

Note: Data were presented as mean ± S.E.M. KO, *n* = 4 giant *ANK2* knockout monkeys, including 2 rhesus and 2 cynomolgus monkeys; WT, *n* = 11 wild-type monkeys, including 5 rhesus and 6 cynomolgus monkeys; FCD, functional connectivity density; Dc, degree centrality; NCp, nodal clustering coefficient.

*, *p* < 0.05; **, *p* < 0.01; ***, *p* < 0.001.

Table S6. Statistical summary and model diagnostics for corrected and nominal MRI/fMRI findings.

Parameter	Regions	<i>P</i> _Shapiro Wilk	<i>P</i> _Brown Forsythe	Cohens d	uncorrected <i>p</i> value	Corrected <i>p</i> value
Surface area	area 24a/b	0.676	0.528	0.448	0.004**	0.100
Surface area	SMA	0.013*	0.990	0.269	0.005**	0.100
Surface area	area 46v/f	0.431	0.667	0.498	0.009**	0.120
Surface area	area 8A	0.407	0.590	0.446	0.017*	0.169
Surface area	area 32	0.122	0.662	0.370	0.027*	0.198
Surface area	area 9	0.089	0.102	0.333	0.031*	0.198
Surface area	area 24c prime	0.498	0.752	-0.374	0.036*	0.200
Surface area	LIP	0.238	0.006**	0.346	0.045*	0.217
Thickness	area 24a/b	0.402	0.964	1.652	<0.001***	<0.001***
Thickness	area 24a/b prime	0.861	0.835	1.026	<0.001***	0.007**
Thickness	area 24c	0.283	0.230	-1.617	0.001**	0.007**
Thickness	area 25	0.339	0.737	1.195	0.003**	0.033*
Thickness	area 32	0.769	0.704	0.822	0.019*	0.147
Gray matter volume	area 24a/b	0.867	0.603	0.651	<0.001***	0.024*
Gray matter volume	area 32	0.930	0.335	0.419	0.006**	0.090
myelination in cortex	M1	0.744	0.262	-1.425	0.036*	0.301
myelination in cortex	Iam/Iapm	0.004**	0.798	-0.888	0.040*	0.301
myelination in subcortex	Hy	0.131	0.318	-2.936	<0.001***	0.004**
myelination in subcortex	Pd	0.935	0.263	-1.463	<0.001***	0.019*
myelination in subcortex	Pu	0.234	0.098	-1.533	0.008**	0.117
myelination in subcortex	Acb	0.040*	0.173	-0.853	0.036*	0.301
subcortex volume	Pd	0.042*	0.838	0.579	0.001**	0.030*
NCp	SMA	0.211	0.706	-0.787	<0.001***	0.002**
NCp	area 3a/b ^a	0.196	0.044*	-0.968	<0.001***	0.013*
NCp	area 25	<0.001***	0.684	0.640	0.001**	0.020*
NCp	area 24c prime	0.510	0.633	-0.684	0.002**	0.022*
NCp	preSMA	0.205	0.188	-0.689	0.003**	0.029*
NCp	area 24a/b	0.003**	0.797	0.671	0.005**	0.034*
NCp	SII	0.530	0.755	-0.580	0.013*	0.072
NCp	PMv	0.042*	0.225	-0.561	0.014*	0.072
NCp	Hy	0.011*	0.034*	-0.493	0.016*	0.072
NCp	areas 1-2	0.215	0.843	-0.595	0.017*	0.072
NCp	M1	0.351	0.583	-0.696	0.018*	0.072

NCp	LIP	0.174	0.517	-0.674	0.023*	0.085
NCp	area 8B	0.005**	0.362	-0.622	0.027*	0.095
Dc	area 7op	0.225	0.063	-1.505	<0.001***	<0.001***
Dc	area 5d ^b	0.546	<0.001***	-1.379	<0.001***	<0.001***
Dc	PEa ^c	0.203	0.011*	-1.205	<0.001***	<0.001***
Dc	Pu	0.195	0.754	0.775	0.002**	0.019*
Dc	area 8A	0.382	0.944	-0.887	0.002**	0.020*
Dc	area 24a/b	0.721	0.215	0.778	0.009**	0.069
Dc	area 3a/b	0.185	0.554	0.599	0.016*	0.101
Dc	area 10	0.516	0.297	0.538	0.018*	0.101
Dc	Acb	0.215	0.039*	0.593	0.024*	0.120
Dc	area 9	0.796	0.885	0.608	0.033*	0.120
Dc	area 46d	0.834	0.935	0.418	0.035*	0.120
Dc	preSMA	0.325	0.494	-0.567	0.035*	0.120
Dc	area 13	0.461	0.003**	0.565	0.035*	0.120
Dc	areas 1-2	0.099	0.174	-0.589	0.037*	0.120
Dc	area 24c	0.549	0.772	0.472	0.044*	0.125
Dc	PMd	0.342	0.094	-0.467	0.045*	0.125
FCD	area 7op	0.499	0.290	-1.061	<0.001***	0.011*
FCD	area 5d	<0.001***	0.058	-0.934	<0.001***	0.011*
FCD	PEa	0.006**	0.490	-0.742	0.007**	0.085
FCD	Acb	0.001**	0.012*	0.685	0.008**	0.085
FCD	area 24a/b	0.100	0.025*	0.761	0.010**	0.088
FCD	Pu	<0.001***	0.914	0.556	0.017*	0.126
FCD	area 14	0.001**	0.331	0.552	0.036*	0.234
FCD	area 10	0.030*	0.084	0.515	0.046*	0.259
intra hemisphere link	par-par	0.060	0.706	-0.738	0.004**	0.025*
intra hemisphere link	sub-fro ^d	0.950	0.014*	0.689	0.011*	0.032*
inter hemisphere link	par-par	0.755	0.307	-0.596	0.025*	0.053
inter hemisphere link	sub-fro	0.651	0.730	0.641	0.026*	0.053
inter hemisphere link	sub-sub	0.976	0.136	0.491	0.026*	0.053
Small worldness	global	0.042*	0.161	1.379	0.007**	0.007**

^a Welch t-test, $p = 0.074$; ^b Welch t-test, $p < 0.001$; ^c Welch t-test, $p < 0.001$; ^d Welch t-test, $p = 0.072$

Note: $P_{\text{ShapiroWilk}}$, p value from the Shapiro-Wilk test of normality; $P_{\text{BrownForsythe}}$, p value from the Brown-Forsythe test of homogeneity of variance; FCD, functional connectivity density; Dc, degree centrality; NCp, nodal clustering coefficient.

*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Table S7 Characteristics and comparison of echocardiography between the giant *ANK2* knockout (KO) and wild-type (WT) monkeys

Variable	Unit	WT (<i>n</i> = 11)	KO (<i>n</i> = 4)	<i>t</i> / <i>U</i>	<i>p</i> value
RVEDD	mm	7.23 ± 0.66	6.89 ± 1.43	20 (<i>U</i>)	0.832
LVEDD	mm	18.28 ± 1.13	19.60 ± 2.90	0.524 (<i>t</i>)	0.609
IVSD	mm	4.38 ± 0.34	4.63 ± 0.85	0.334 (<i>t</i>)	0.744
LVPWD	mm	4.71 ± 0.24	4.63 ± 0.33	0.162 (<i>t</i>)	0.874
LVESD	mm	12.31 ± 0.88	12.93 ± 2.18	0.323 (<i>t</i>)	0.752
IVSS	mm	5.65 ± 0.39	5.69 ± 0.85	0.054 (<i>t</i>)	0.957
LVPWS	mm	6.02 ± 0.30	5.73 ± 0.65	0.452 (<i>t</i>)	0.659
RAD	mm	13.81 ± 0.72	13.14 ± 2.41	0.369 (<i>t</i>)	0.718
LAD	mm	11.75 ± 0.77	10.79 ± 1.24	0.646 (<i>t</i>)	0.529
AOD	mm	10.17 ± 0.36	10.06 ± 0.93	0.140 (<i>t</i>)	0.891
AVV	m/s	1.13 ± 0.05	0.94 ± 0.05	2.180 (<i>t</i>)	0.048*
PAD	mm	9.69 ± 0.30	9.56 ± 0.74	0.208 (<i>t</i>)	0.838
PVV	m/s	1.00 ± 0.04	1.03 ± 0.10	0.452 (<i>t</i>)	0.659
FS	%	33.00 ± 1.26	34.33 ± 1.76	0.568 (<i>t</i>)	0.580
EF	%	64.67 ± 1.93	66.42 ± 2.74	0.484 (<i>t</i>)	0.637
E-wave velocity	m/s	87.06 ± 7.19	79.50 ± 5.18	0.389 (<i>t</i>)	0.704
A-wave velocity	m/s	86.64 ± 5.28	90.25 ± 6.59	0.545 (<i>t</i>)	0.595
E/A ratio	%	1.03 ± 0.09	0.88 ± 0.02	21 (<i>U</i>)	0.930

Note: Data were presented as mean ± S.E.M. KO, *n* = 4 giant *ANK2* knockout monkeys, including 2 rhesus and 2 cynomolgus monkeys; WT, *n* = 11 wild-type monkeys, including 5 rhesus and 6 cynomolgus monkeys. The *p* values were defined by the Student's *t* tests or the Mann-Whitney *U* test. *, *p* < 0.05; *t*: Student's *t* statistic value; *U*: Mann-Whitney *U* statistic value.

RVEDD, right ventricle end-diastolic diameter; LVEDD, left ventricle end-diastolic diameter; IVSD, interventricular septal thickness in diastole; LVPWD, left ventricular posterior wall thickness in diastole; LVESD, left ventricular end- systolic dimension; IVSS, interventricular septal thickness in systole; LVPWS, left ventricular posterior wall thickness in systole; RAD, Right atrial diameter; LAD, left atrial diameter; AOD, aorta diameter; AVV, aortic valve velocity; PAD, Pulmonary artery diameter; PVV, pulmonary valve velocity; FS, fractional shortening; EF, ejection fraction.

Table S8 Hematological parameters with non-significant difference between the giant *ANK2* knockout (KO) and wild-type (WT) monkeys

Variable	Unit	WT	KO	<i>t</i> / <i>U</i>	<i>p</i> value
Blood biochemistry test (BCT, WT group, n=78; KO group, n = 4)					
AST	U/L	63.44 ± 7.95	45.10 ± 7.75	132.5 (<i>U</i>)	0.628
ALP	U/L	375.92 ± 17.83	271.65 ± 70.11	100 (<i>U</i>)	0.243
GGT	U/L	91.91 ± 5.03	81.70 ± 11.46	135 (<i>U</i>)	0.668
CRE2	μmol/L	117.06 ± 2.76	112.05 ± 11.15	137 (<i>U</i>)	0.698
CK	U/L	333.68 ± 36.94	348.75 ± 241.83	108 (<i>U</i>)	0.318
GLU	mmol/L	6.20 ± 0.26	4.63 ± 0.35	1.360 (<i>t</i>)	0.178
Complete blood count (CBC, WT group, n=11; KO group, n = 4)					
WBC	10 ⁹ /L	11.17 ± 0.79	11.18 ± 0.91	0.006 (<i>t</i>)	0.995
Lym#	10 ⁹ /L	5.67 ± 0.43	5.15 ± 0.32	188 (<i>U</i>)	0.806
Mon#	10 ⁹ /L	0.63 ± 0.06	0.72 ± 0.07	0.816 (<i>t</i>)	0.419
Gran#	10 ⁹ /L	4.87 ± 0.40	5.32 ± 0.85	189 (<i>U</i>)	0.825
Lym%	%	50.99 ± 1.65	48.8 ± 3.98	0.606 (<i>t</i>)	0.547
Mon%	%	5.62 ± 0.30	6.42 ± 0.49	1.358 (<i>t</i>)	0.182
Gran%	%	43.38 ± 1.62	44.78 ± 4.00	0.390 (<i>t</i>)	0.699
HGB	g/L	147.12 ± 1.05	148.67 ± 2.10	0.717 (<i>t</i>)	0.477
HCT	%	44.13 ± 0.75	45.34 ± 0.73	171.5 (<i>U</i>)	0.505
MCH	pg	24.4 ± 0.17	23.92 ± 0.26	1.507 (<i>t</i>)	0.139
MCHC	g/L	329 ± 1.86	327.67 ± 3.26	0.364 (<i>t</i>)	0.717
RDW	%	12.3 ± 0.23	12.48 ± 0.34	171 (<i>U</i>)	0.497
PLT	10 ⁹ /L	407.58 ± 16.94	417.58 ± 18.42	0.330 (<i>t</i>)	0.743
MPV	fL	9.75 ± 0.21	9.83 ± 0.38	0.176 (<i>t</i>)	0.861
PDW	%	15.47 ± 0.10	15.33 ± 0.14	170.5 (<i>U</i>)	0.487
PCT	%	0.4 ± 0.02	0.40 ± 0.01	0.287 (<i>t</i>)	0.776

Note: Data were presented as mean ± S.E.M. KO group, *n* = 4 giant *ANK2* knockout monkeys, including 2 rhesus and 2 cynomolgus monkeys; WT group for BCT, *n* = 78 wild-type monkeys, including 58 rhesus and 20 cynomolgus monkeys; WT group for CBC, *n* = 11 wild-type monkeys, including 5 rhesus and 6 cynomolgus monkeys. The *p* values were defined by the Student's *t* tests or the Mann-Whitney *U* test. *t*: Student's *t* statistic value; *U*: Mann-Whitney *U* statistic value.

In BCT, AST, aspartate amino transferase; ALP, alkaline phosphatase; GGT, γ-glutamyl transferase; CRE2, creatinine; CK, creatine kinase; GLU, blood glucose. In CBC, WBC, white blood cell; Lym#, number of lymphocytes; Mon#, number of monocytes; Gran#, number of granulocytes; Lym%, proportion of lymphocyte; Mon%, proportion of monocytes; Gran%, proportion of granulocytes; HGB, hemoglobulin; HCT, hematocrit; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red blood cell

volume distribution width; PLT, platelet; MPV, mean platelet volume; PDW, platelet volume distribution width; PCT, plateletcrit

Table S9 Significantly altered lipid species in CSF and serum between the giant *ANK2* knockout (KO) and wild-type (WT) monkeys

Lipid species	Class	Calibrated m/z	Fold change	<i>U</i>	<i>p</i> value
CSF					
PI(15:0/17:0)	PI	809.5/241.2	6.39	1.5	0.0010
TG(52:4)-FA16:0	TG	872.8/599.5	5.31	5	0.0060
TG(54:3)-FA18:0	TG	902.8/601.5	5.31	5	0.0060
TG(54:3)-FA18:2	TG	902.8/605.5	5.08	4	0.0040
TG(52:4)-FA18:2	TG	872.8/575.5	4.96	5	0.0060
TG(52:3)-FA16:0	TG	874.8/601.5	4.83	5	0.0060
TG(52:5)-FA18:2	TG	870.8/573.5	4.77	3	0.0023
TG(52:3)-FA18:2	TG	874.8/577.5	4.56	6	0.0090
TG(54:5)-FA18:1	TG	898.8/599.5	4.46	3	0.0023
TG(54:5)-FA18:2	TG	898.8/601.5	4.43	3	0.0023
TG(54:4)-FA18:0	TG	900.8/599.5	4.37	3	0.0023
TG(54:4)-FA18:1	TG	900.8/601.5	4.37	5	0.0060
TG(54:4)-FA18:2	TG	900.8/603.5	4.35	5	0.0060
TG(56:4)-FA20:1	TG	928.8/601.5	4.35	6	0.0090
TG(50:4)-FA14:0	TG	844.6/599.4	4.32	4	0.0040
TG(52:5)-FA16:1	TG	870.8/599.5	4.28	5	0.0060
TG(56:5)-FA18:2	TG	926.8/629.5	4.26	11	0.0404
TG(52:5)-FA16:0	TG	870.8/597.5	4.20	3	0.0023
TG(52:4)-FA18:3	TG	872.8/577.5	4.13	6	0.0090
TG(52:4)-FA18:1	TG	872.8/573.5	4.13	6	0.0090
TG(50:2)-FA18:2	TG	848.8/551.5	4.06	5	0.0060
TG(52:5)-FA18:3	TG	870.8/575.5	4.02	5	0.0060
TG(50:3)-FA16:0	TG	846.8/573.5	3.83	6	0.0090
TG(52:6)-FA16:1	TG	868.8/597.5	3.80	1	0.0007
TG(52:3)-FA18:0	TG	874.8/573.5	3.77	6	0.0090
TG(52:3)-FA18:1	TG	874.8/575.5	3.61	6	0.0090
TG(56:4)-FA18:2	TG	928.8/631.5	3.58	9	0.0237
TG(52:5)-FA18:1	TG	870.8/571.5	3.55	3	0.0023
TG(54:6)-FA18:1	TG	896.8/597.5	3.34	3	0.0023
TG(56:5)-FA18:1	TG	926.8/627.5	3.33	8	0.0177
TG(54:6)-FA18:3	TG	896.8/601.5	3.32	3	0.0023
TG(52:6)-FA18:2	TG	868.8/571.5	3.30	4	0.0040
TG(56:5)-FA20:1	TG	926.8/599.5	3.29	7	0.0127
TG(56:8)-FA22:6	TG	920.8/575.5	3.27	8	0.0177
TG(52:4)-FA16:1	TG	872.8/601.5	3.27	6	0.0090
TG(54:3)-FA20:1	TG	902.8/575.5	3.19	6	0.0090
TG(52:6)-FA18:3	TG	868.8/573.5	3.16	0	0.0003
TG(54:4)-FA18:3	TG	900.8/605.5	3.16	6	0.0090
TG(53:4)-FA17:0	TG	886.8/599.5	3.14	9	0.0237

Lipid species	Class	Calibrated m/z	Fold change	<i>U</i>	<i>p</i> value
TG(54:4)-FA16:0	TG	900.8/627.5	3.13	9	0.0237
TG(54:5)-FA18:3	TG	898.8/603.5	3.11	4	0.0040
TG(54:2)-FA18:2	TG	904.8/607.5	3.08	5	0.0060
TG(50:3)-FA14:0	TG	846.8/601.6	3.04	5	0.0060
TG(54:3)-FA16:0	TG	902.8/629.5	3.01	6	0.0090
TG(54:6)-FA18:2	TG	896.8/599.5	2.97	3	0.0023
TG(52:2)-FA18:2	TG	876.8/579.5	2.92	5	0.0060
TG(56:6)-FA18:1	TG	924.8/625.5	2.91	7	0.0127
TG(50:2)-FA16:0	TG	848.8/575.5	2.91	6	0.0090
TG(56:4)-FA18:1	TG	928.8/629.5	2.90	3	0.0023
TG(50:4)-FA18:2	TG	844.6/547.3	2.87	5	0.0060
TG(54:5)-FA16:0	TG	898.8/625.5	2.86	5	0.0060
TG(54:5)-FA18:0	TG	898.8/597.5	2.86	7	0.0127
TG(54:2)-FA18:0	TG	904.8/603.5	2.86	8	0.0177
TG(56:3)-FA20:0	TG	930.8/601.5	2.86	11.5	0.0419
TG(50:3)-FA18:2	TG	846.8/549.5	2.81	6	0.0090
TG(52:2)-FA16:0	TG	876.8/603.5	2.80	6	0.0090
TG(50:4)-FA16:0	TG	844.6/571.3	2.79	7	0.0127
TG(52:2)-FA18:0	TG	876.8/575.5	2.79	7	0.0127
TG(54:3)-FA18:1	TG	902.8/603.5	2.71	8	0.0177
TG(54:4)-FA20:2	TG	900.8/575.5	2.70	8	0.0177
TG(54:2)-FA18:1	TG	904.8/605.5	2.54	9	0.0237
TG(50:5)-FA18:2	TG	842.6/545.3	2.52	6	0.0089
TG(53:4)-FA18:2	TG	886.8/589.5	2.48	8	0.0177
TG(50:3)-FA18:3	TG	846.8/551.5	2.47	8	0.0177
TG(56:7)-FA22:6	TG	922.8/577.5	2.44	8	0.0177
TG(54:4)-FA20:1	TG	900.8/573.5	2.41	5	0.0055
TG(56:3)-FA18:1	TG	930.8/631.5	2.40	6	0.0090
TG(50:5)-FA14:0	TG	842.6/597.4	2.38	9	0.0237
TG(52:2)-FA18:1	TG	876.8/577.5	2.37	6	0.0090
TG(56:3)-FA20:1	TG	930.8/603.5	2.35	11	0.0404
TG(53:3)-FA17:0	TG	888.8/601.5	2.33	3	0.0023
TG(54:7)-FA18:2	TG	894.8/597.5	2.33	2	0.0013
DG(18:0/20:4)	DG	662.6/341.3	2.32	6	0.0090
TG(58:8)-FA22:6	TG	948.8/603.5	2.31	6	0.0090
TG(54:5)-FA20:4	TG	898.8/577.5	2.31	9	0.0237
TG(54:7)-FA18:3	TG	894.8/599.5	2.30	3	0.0023
TG(56:7)-FA18:1	TG	922.8/623.5	2.28	11	0.0393
TG(56:9)-FA22:6	TG	918.8/573.5	2.27	5	0.0058
TG(56:6)-FA22:4	TG	924.8/575.5	2.25	6	0.0089
TG(52:8)-FA18:2	TG	864.8/567.5	2.24	7.5	0.0135
PE(O-16:0/18:2)	PE-O	700.5/279.2	2.23	6	0.0090
PE(18:2/20:4)	PE	762.5/303.2	2.22	11	0.0404

Lipid species	Class	Calibrated m/z	Fold change	<i>U</i>	<i>p</i> value
TG(55:4)-FA18:2	TG	914.8/617.5	2.17	9	0.0237
PE(18:1/20:1)	PE	770.6/309.3	2.17	3	0.0023
PE(18:2/18:2)	PE	738.5/279.2	2.16	11	0.0404
TG(56:2)-FA20:1	TG	932.9/605.6	2.16	6	0.0090
PE(18:1/18:2)	PE	740.5/279.2	2.15	11	0.0404
DG(18:0/22:4)	DG	690.6/341.3	2.14	10	0.0314
TG(56:6)-FA20:4	TG	924.8/603.5	2.13	10	0.0314
PE(16:0/18:2)	PE	714.5/279.2	2.11	9	0.0237
TG(54:6)-FA22:6	TG	896.8/551.5	2.10	7	0.0127
TG(48:2)-FA18:2	TG	820.7/523.5	2.04	5	0.0060
TG(54:5)-FA20:3	TG	898.8/575.5	2.04	11	0.0404
TG(50:4)-FA18:3	TG	844.6/549.3	2.03	9	0.0237
TG(54:3)-FA20:2	TG	902.8/577.5	2.02	10	0.0314
TG(53:3)-FA18:2	TG	888.8/591.5	2.00	8	0.0177
TG(56:2)-FA20:0	TG	932.9/603.6	1.97	11	0.0404
TG(54:2)-FA16:0	TG	904.8/631.5	1.95	10	0.0314
TG(56:3)-FA16:0	TG	930.8/657.5	1.94	10	0.0314
PE(17:0/18:2)	PE	728.5/269.2	1.82	11	0.0404
TG(50:1)-FA16:0	TG	850.8/577.5	1.80	6	0.0090
TG(58:3)-FA18:1	TG	958.9/659.6	1.78	8	0.0177
PE(16:0/18:1)	PE	716.5/281.2	1.74	6	0.0090
TG(48:3)-FA14:0	TG	818.7/573.5	1.74	10	0.0314
TG(50:4)-FA18:1	TG	844.6/545.3	1.74	6	0.0090
ChE(22:2)	ChE	722.7/369.4	1.72	8	0.0177
TG(52:1)-FA16:0	TG	878.8/605.5	1.72	7	0.0127
DG(18:2/20:0)	DG	666.6/337.3	1.70	11	0.0404
PE(18:0/18:1)	PE	744.6/281.2	1.70	9	0.0237
TG(52:6)-FA16:0	TG	868.8/595.5	1.69	11	0.0404
TG(52:3)-FA16:1	TG	874.8/603.5	1.68	6	0.0090
TG(50:1)-FA18:1	TG	850.8/551.5	1.68	6	0.0090
TG(52:1)-FA18:0	TG	878.8/577.5	1.63	7	0.0127
TG(52:3)-FA20:1	TG	874.8/547.5	1.62	8	0.0177
TG(54:4)-FA20:3	TG	900.8/577.5	1.60	11	0.0404
TG(54:1)-FA18:1	TG	906.8/607.5	1.60	10	0.0314
DG(16:0/22:4)	DG	662.6/313.3	1.59	7	0.0127
DG(18:0/20:0)	DG	670.6/341.3	1.59	11	0.0404
TG(55:4)-FA18:1	TG	914.8/615.5	1.57	9	0.0237
TG(52:1)-FA18:1	TG	878.8/579.5	1.53	7	0.0127
TG(50:2)-FA14:0	TG	848.8/603.6	1.52	10	0.0314
TG(52:2)-FA16:1	TG	876.8/605.5	1.52	6	0.0090
PE(16:0/16:0)	PE	690.5/255.2	1.51	10	0.0314
PC(15:0/18:3)	PC	800.5/241.2	0.66	11	0.0404
TG(56:7)-FA16:0	TG	922.8/649.5	0.60	11	0.0393

Lipid species	Class	Calibrated m/z	Fold change	<i>U</i>	<i>p</i> value
TG(58:6)-FA18:1	TG	952.8/653.5	0.40	11.5	0.0428
Serum					
PG(16:0/16:1)	PG	719.5/253.2	3.23	10.5	0.0326
TG(52:8)-FA16:1	TG	864.8/593.5	2.61	11.5	0.0424
PS(18:0/22:6)	PS	834.5/327.2	1.97	7	0.0127
PE(P-18:1/20:0)	PE-P	758.5/369.3	1.65	10.5	0.0338
PC(20:0/16:1)	PC	846.6/253.2	1.63	11.5	0.0426
MGDG(18:1/18:1)	MGDG	800.6/339.3	1.63	8	0.0177
DGDG(18:3/20:1)	DGDG	986.7/367.3	0.30	10	0.0291
ChE(16:0)	ChE	642.6/369.4	0.26	3	0.0023

Note: Fold change > 1.5 indicates up-regulation and < 2/3 indicates down-regulation. The *p* values were determined by the Mann-Whitney U test. *U*: Mann-Whitney U statistic value. KO group, *n* = 4, including 2 rhesus monkeys and 2 cynomolgus monkeys; WT group, *n* = 17, including 11 rhesus monkeys and 6 cynomolgus monkeys.

PI, phosphatidylinositol; TG, triglyceride; PE, phosphatidylethanolamine; DG, diacylglycerols; PC, phosphatidylcholine; PG, phosphatidylglycerol; PS, phosphatidylserine; PE-O, alkyl-phosphatidylethanolamine; PE-P, alkenyl-phosphatidylethanolamine; MGDG, monogalactosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; ChE, cholesterol Ester