The mechanisms that extend lifespan in humans are poorly understood. Here we show that extended longevity in humans is associated with a distinct transcriptome signature in the cerebral cortex that is characterized by downregulation of genes related to neural excitation and synaptic function. In Caenorhabditis elegans, neural excitation increases with age and inhibition of excitation globally, or in glutamatergic or cholinergic neurons, increases longevity. Furthermore, longevity is dynamically regulated by the excitatory–inhibitory balance of neural circuits. The transcription factor REST is upregulated in humans with extended longevity and represses excitation–related genes. Notably, REST–deficient mice exhibit increased cortical activity and neuronal excitability during ageing. Similarly, loss–of–function mutations in the C. elegans REST orthologue genes spr–3 and spr–4 elevate neural excitation and reduce the lifespan of long–lived daf–2 mutants. In wild–type worms, overexpression of spr–4 suppresses excitation and extends lifespan. REST, SPR–3, SPR–4 and reduced excitation activate the longevity–associated transcription factors FOXO1 and DAF–16 in mammals and worms, respectively. These findings reveal a conserved mechanism of ageing that is mediated by neural circuit activity and regulated by REST.

Studies in invertebrate and mammalian models suggest that the nervous system plays a role in the regulation of ageing1–2. In the nematode C. elegans, ablation of specific sensory or neurosecretory neurons alters lifespan3–4, and lifespan extension from reduced insulin/IGF–like signalling can be reversed by restoring function specifically in neurons5. However, whether the activity state of the nervous system affects the ageing process is unclear. Here we describe a conserved mechanism of ageing that is mediated by global neural activity and regulated by REST.

Neural excitation and longevity

Previous studies of the ageing human brain have shown dynamic changes in gene expression that distinguish young adults from the ageing population6. However, the recent expansion of data from ageing human cohorts has enabled partitioning of the ageing population into subgroups based on transcriptome profiling. To gain insight into changes in gene expression in the brain that are associated with extended human longevity, we analysed RNA sequencing (RNA–seq)9,10 and microarray data11 from the frontal cortex of aged individuals with intact cognitive function in three cohorts: ROSMAP, CommonMind Consortium (CMC), and Gibbs. We compared age–associated genes between all individuals and derived Pearson correlation coefficients. Hierarchical clustering suggested that the ageing population partitioned into three groups (Extended Data Fig. 1a–c). The most significant changes associated with the extended longevity groups (≥85 years versus ≤80 years of age) were downregulation of genes related to neural excitation and synaptic function, and upregulation of genes involved in immune function (Fig. 1a–d, Extended Data Fig. 1d–f, Supplementary Tables 1–6). This did not change after adjusting for amyloid deposits and neurofibrillary tangles in the ROSMAP cohort (data not shown). Meta–analysis of gene ontology (GO) enrichment in each cohort indicated that terms related to excitatory, but not inhibitory, synaptic transmission were enriched in the downregulated genes (Extended Data Fig. 1g, Supplementary Table 7). These results suggest that extended human longevity may be associated with reduced excitatory neurotransmission.

To explore the neural regulation of longevity, we used C. elegans, a well–established model system of ageing. We monitored neural excitation in C. elegans by GCaMP calcium imaging in the glutamatergic ASH neurons12. In wild–type worms, we observed rapid, transient pulses of GCaMP fluorescence indicative of neuronal excitation (Supplementary Video 1). Calcium influx in ASH neurons increased during normal ageing from adult day 1–2 to day 12–16 (Fig. 1e). To determine the effect of decreasing calcium influx on lifespan, worms were treated with nemadipine, an inhibitor of L–type calcium channels that reduces neural excitation (Fig. 1f). Continuous treatment with nemadipine beginning at adult day 1 extended lifespan (Fig. 1g). Moreover, incubation of worms with ivermectin, an agonist of invertebrate glutamate–gated chloride channels, suppressed neural excitation and resulted in a dose–dependent extension of mean lifespan (Fig. 1h, i). Nemadipine and ivermectin also extended lifespan when administered at day 8, when feeding activity has largely abated (Extended Data Fig. 2a), suggesting that the drugs do not act through caloric restriction. Furthermore, worm motility was preserved (Extended Data Fig. 2b, Supplementary Videos 4–6). These results suggest that global inhibition of neural excitation extends lifespan in C. elegans.

To explore the neural systems that mediate lifespan, we expressed a transgenic Drosophila histamine–gated chloride channel (HisClI) in different neuronal populations in C. elegans13. Addition of histamine, which is not endogenously produced by worms, activates HisClI and inhibits neural excitation. First, we expressed HisClI under the control of a pan–neuronal promoter. Continuous incubation with histamine beginning on adult day 1 or day 8 extended mean lifespan (Extended Data Fig. 3a, b, i). Histamine had no effect on the lifespan of wild–type worms that did not express HisClI (Supplementary Table 22).
We next expressed the HisCl1 channel in glutamatergic and cholinergic neurons, the major excitatory neuronal populations in C. elegans. Repression of excitation in either population robustly extended lifespan whether initiated at adult day 1 or day 9 (Extended Data Fig. 3c–f, i). Expression of HisCl1 in γ-aminobutyric acid (GABA)ergic neurons using an *unc-47* driver extended lifespan when initiated at adult day 1, but reduced lifespan when initiated at day 8 (Extended Data Fig. 3g–i). GCaMP imaging showed that addition of histamine to *unc-47*HisCl1 worms at day 1 resulted in marked and persistent suppression of excitation in ASH neurons, which was not observed after addition at day 8 (Extended Data Fig. 3). Thus, blockade of GABAergic neurotransmission early in adult life may result in compensatory downregulation of excitation in other neuronal populations. Together, these results suggest that continuous or late-life repression of neural excitation in multiple neuronal cell populations extends lifespan in *C. elegans*.

To explore the effect of hyperexcitation on lifespan, we suppressed GABAergic neurotransmission by using RNA-mediated inhibition (RNAi), which would be predicted to be less extensive than histamine/HisCl1-mediated blockade. When worms were treated with RNAi against the GABA vesicular transporter *unc-47*, there was a robust increase in excitation in ASH neurons and a reduction in lifespan (Extended Data Fig. 4a, b). Thus, the effects of neurotransmission on lifespan are bidirectional; lifespan is extended by reducing excitation and shortened by increasing excitation.

Neural activity can regulate neuropeptide secretion. To investigate the role of neuropeptide signalling in lifespan regulation, we evaluated worms in which the function of the EGL-3 proprotein convertase was blocked by an egl-3 mutation or egl-3 RNAi. The egl-3 mutant exhibited robust lifespan extension (Extended Data Fig. 4c). Lifespan was also extended in worms treated with egl-3 RNAi (Extended Data Fig. 4d), consistent with previous results14. Similar extension of lifespan was observed in the glutamatergic loss-of-function *eat-4* mutant and the synaptic transmission *unc-13* mutant (Extended Data Fig. 4c). These results suggest that both synaptic neurotransmission and peptideergic signalling contribute to the regulation of lifespan.

**REST modulates excitation in the ageing brain**

We have previously demonstrated that the transcriptional represor REST is induced in the ageing brain15. Genes that were downregulated in the brain in individuals with extended longevity were enriched for the canonical REST RE1 motif in all three ageing cohorts (ROSMAP, *P* = 5 × 10−12; CMC, *P* = 8 × 10−4; Gibbs, *P* = 1 × 10−2; Supplementary Tables 1, 3, 5). Moreover, unbiased analysis by chromatin immunoprecipitation and sequencing (ChIP–seq) showed that REST was the most strongly implicated transcription factor in multiple ENCODE datasets (Supplementary Tables 8–11). Furthermore, the downregulated gene set was highly enriched for neuronal REST target genes (Supplementary Table 12). Expression of these downregulated genes, as well as an index of synaptic gene expression, were inversely related to expression of REST mRNA (Fig. 2a, b, Extended Data Fig. 5a, b). Furthermore, levels of REST in nuclei were elevated in prefrontal cortical neurons in centenarians relative to individuals who...
were 70–80 years of age (Fig. 2c). Although expression of REST mRNA is upregulated in the brain during ageing15, increased REST mRNA expression did not distinguish between the extended longevity and normal ageing groups based on RNA-seq (Supplementary Tables 1, 3). However, for a given level of expression of REST mRNA, there is greater gene downregulation in the extended longevity group (Extended Data Fig. 5c, d). These results suggest that REST repressor function is upregulated in the brain in individuals with extended longevity, resulting in downregulation of genes that mediate excitation and synaptic function.

To assess the role of REST as a modulator of neural activity in the ageing mammalian brain, we examined the uptake of fluorodeoxyglucose (FDG) by positron emission tomography and computerized tomography (PET–CT) in the brains of 18-month-old Nestin-Cre:Restlox/lox (REST conditional knockout (cKO)) mice and littermate controls15 (Extended Data Fig. 5e). REST cKO mice showed elevated cortical 18F-FDG uptake, indicative of increased neural activity (Fig. 2d, e). Previous studies suggest that REST can modulate excitability in mouse models of epilepsy induced by kindling or kainate16,17. To assess excitability during ageing, we performed electroencephalographic (EEG) recordings of REST cKO mice (Supplementary Table 13). Intermittent epileptiform discharges were more frequent in 22.5- to 23-month-old REST cKO mice than in controls (Fig. 2f). Furthermore, challenge with the GABA antagonist pentylentetrazol (PTZ) increased seizure duration in REST cKO mice relative to controls (Fig. 2g), with a trend towards increased mortality (Extended Data Fig. 5f). These results suggest that REST globally represses neural activity and prevents hyperexcitation in the ageing brain.

C. elegans REST orthologues regulate longevity
The C. elegans gene spr-4 encodes a structural and functional orthologue of mammalian REST that protects against toxic stressors, such as reactive oxygen species and amyloid-β protein15. To determine whether spr-4 modulates lifespan, we induced endogenous expression of spr-4 by using the RNA-guidedendonuclease Cas9 as a programmable transcription factor18. A nuclease-deficient variant of Cas9 (dCas9) was fused to the transcriptional activator VP16 (dCas9:VP64) and stably introduced into C. elegans together with four small guide RNAs (sgRNAs) targeting the spr-4 promoter. This resulted in a modest elevation in spr-4 mRNA and protein expression, and a significant increase in mean lifespan (Extended Data Fig. 6a–d). Expression of dCas9:VP64 and spr-4 sgRNAs in worms with the loss-of-function allele spr-4 (tm465) did not affect lifespan, suggesting specificity for spr-4 (Extended Data Fig. 6e). Moreover, overexpression of spr-4 robustly reduced excitation in ASH neurons (Extended Data Fig. 6f). Thus, SPR-4 both represses neural excitation and extends lifespan.

The forkhead transcription factor DAF-16 is the central downstream target of the DAF-2–insulin/IGF-like signalling pathway that regulates lifespan in C. elegans. RNAi-mediated knockdown of daf-16 prevented extension of lifespan by overexpression of spr-4 (Extended Data Fig. 7a). Furthermore, extension of lifespan by the neural excitation inhibitors nemadipine and ivermectin was also dependent on daf-16, and ivermectin elevates both total and nuclear levels of DAF-16 (Extended Data Fig. 7b–f). Thus, DAF-16 mediates extension of lifespan by spr-4 and neural suppression.

To further explore the effects of REST orthologues on the DAF-2–DAF-16 signalling pathway, we performed daf-2 RNAi in wild-type worms, spr-3 and spr-4 loss-of-function mutants, and an spr-4;spr-3 double mutant. As previously shown19, daf-2 RNAi extends lifespan by about 50% in wild-type worms. Mutations in spr-3, spr-4, or both reduced the extension of lifespan by daf-2 RNAi (Extended Data Fig. 8a). Mutations in spr-3 and spr-4 also reduced the lifespan extension associated with the loss-of-function daf-2(e1370) allele; the greatest reduction occurred in the spr-3;spr-4;daf-2 triple mutant (Fig. 3a). The spr-3 and spr-4 mutations did not affect lifespan in a wild-type background (Extended Data Fig. 8b), in contrast to a previous report that suggested that spr-3 mutations altered lifespan20. These results suggest that spr-3 and spr-4 contribute to the regulation of lifespan by the insulin/IGF-like signalling pathway in worms.

We next investigated whether SPR-3 and SPR-4 function in neurons to regulate lifespan. To address this question, we used a C. elegans line in which RNAi was abolished by deletion of the double-stranded RNA transporter SID-1, but restored specifically in neurons by a sid-1 transgene driven by a neuron-specific promoter21. These alleles were crossed into the daf-2(e1370) mutant background, and RNAi-mediated knockdown of both spr-3 and spr-4 was performed. Neuron-targeted knockdown of spr-3 and spr-4 significantly reduced lifespan (Fig. 3b, Extended Data Fig. 8c). Thus, neuronal expression of spr-3 and spr-4 contributes to lifespan extension in the daf-2 mutant.

SPR-3 and SPR-4 regulate neural excitation
To gain further insight into the effects of SPR-3 and SPR-4 on DAF-2 function and lifespan, we performed RNA-seq on wild-type worms and...
versus a neuropeptide systems to extend lifespan in mutant worms. daf-2 SPR-3 and SPR-4 suppress multiple neurotransmitter and neuropeptide systems to extend lifespan in mutant worms. daf-2 SPR-3 and SPR-4 suppress multiple neurotransmitter and neuropeptide systems to extend lifespan in mutant worms. The comparison of triple mutant spr-4;spr-3;daf-2 worms with single mutant daf-2 worms was notable for highly significant changes in the transcriptome that were enriched for GO terms related to neural excitation, signalling and synaptic function (Fig. 3c, Supplementary Table 15). Furthermore, gene expression changes in daf-2 mutants and spr-4;spr-3 mutants overlapped (Extended Data Fig. 8e), and genes that were downregulated in daf-2 mutants but upregulated in the spr-4;spr-3;daf-2 triple mutants were enriched for GO terms related to neural excitation (Supplementary Table 16). These results suggest that repression of neuronal genes is a conserved regulatory feature of REST and its worm orthologues.

A central question is whether SPR-3 and SPR-4 affect lifespan by suppressing neural excitation. GCaMP calcium imaging showed that neural excitation was strongly suppressed in daf-2 mutants, both in young adult worms and during ageing (Fig. 4a, b, Extended Data Fig. 8f, Supplementary Videos 1–3). The spr-4;spr-3 mutations partially restored neural excitation in daf-2 mutants (Fig. 4a, b), but did not increase excitation in wild-type worms (Extended Data Fig. 8g). Suppression of excitation in daf-2 mutants was not mediated by neuronal DAF-16 (Extended Data Fig. 8h). However, inhibition of neural excitation with ivermectin reversed the lifespan-shortening effect of spr-4;spr-3 mutations in daf-2 mutant worms (Fig. 4c). Thus, SPR-3 and SPR-4 contribute to the extreme longevity of daf-2 mutant worms by repressing neural excitation.

**Fig. 4** | SPR-3 and SPR-4 suppress multiple neurotransmitter and neuropeptide systems to extend lifespan in daf-2 mutant worms. a, Neural excitation is suppressed in daf-2 mutants and partially restored by spr-4;spr-3 mutations. GCaMP imaging was performed in ASH neurons. Shown is the fraction of worms with at least one firing event in a 2-min recording. Mean ± s.e.m., n = 4–5 independent experiments. **P = 7.9 × 10⁻⁷ (daf-2 versus wild-type), P = 1.5 × 10⁻⁴ (spr-4;spr-3; daf-2 versus daf-2); P = 0.0011 (spr-4;spr-3;daf-2 versus wild-type); ANOVA with post hoc Tukey test. b, Quantification of GCaMP fluorescence changes in day 2 worms: wild-type, n = 53; daf-2, n = 25; spr-4;spr-3;daf-2, n = 26. **P = 3.1 × 10⁻⁴ (daf-2 versus wild-type), P = 1.5 × 10⁻³ (spr-4;spr-3;daf-2 versus daf-2). *P = 0.018 (spr-4;spr-3;daf-2 versus wild-type); Mann–Whitney U-test with multiple testing correction by Holm’s method. c, Inhibition of neural excitation by ivermectin (+Ive, 10 pg ml⁻¹) reverses lifespan reduction by spr-4;spr-3 mutations in daf-2 mutant worms (P = 1.1 × 10⁻⁶ (spr-4;spr-3;daf-2 +Ive versus –Ive)); daf-2 –Ive, n = 53 worms; daf-2 +Ive, n = 55; spr-4;spr-3;daf-2 –Ive, n = 95; spr-4;spr-3;daf-2 +Ive, n = 69; WT –Ive, n = 64. d, Multiple neurotransmitter and neuropeptide signalling systems contribute to the effects of spr-4;spr-3 mutations on longevity. Change in lifespan of spr-4;spr-3;daf-2 triple mutant and daf-2 single mutant worms following neuronal RNAi of the indicated genes relative to empty vector control RNAi. RNAi was targeted to neurons as described in Fig. 3b. **P < 0.05, ***P < 0.01, Student’s t-test. n = 3 independent experiments per group. Individual statistics are in Supplementary Table 22.
REST, FOXO1 and neural excitation

We next investigated whether the association between REST, neural excitation and human longevity (Fig. 2a–c) might be mediated by a mammalian forkhead transcription factor orthologue to C. elegans DAF-16. REST has been shown to regulate the expression of FOXO1 in SH-SY5Y neuroblastoma cells. In the human brain, expression of REST mRNA was positively correlated with expression of FOXO1 mRNA, but did not correlate with the expression of other FOXO family members (Fig. 5a, Extended Data Fig. 10a). Furthermore, REST and FOXO1 co-localized in neurons of the ageing human prefrontal cortex (Extended Data Fig. 10b), and nuclear levels of REST and FOXO1 were strongly positively correlated in all age groups (Fig. 5b).

To determine whether REST regulates FOXO1 expression in the brain, we examined Rest KO and littermate control mice. FOXO1 localized predominantly to cortical neurons, and showed an age-related increase in 18-month-old relative to 9-month-old control mice (Fig. 5c). Age-dependent induction of nuclear FOXO1 was abolished in Rest cKO mice (Fig. 5c). Thus, regulation of forkhead transcription factors is a conserved feature of REST and its C. elegans orthologues.

To explore the role of neural excitation in regulation of FOXO1, we treated primary mouse cortical neuronal cultures with kynurenic acid (a broad-spectrum glutamate receptor antagonist) or with either 2-amino-5-phosphonovalerate (AP5) or 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline (NBQX) (antagonists for the NMDA (N-methyl-D-aspartate) and AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid)/kainate subtypes of glutamate receptor, respectively). Kynurenic acid and NBQX significantly increased nuclear and total levels of FOXO1 (Extended Data Fig. 10c). Thus, FOXO1 is regulated by glutamatergic signalling in mammalian cortical neurons, paralleling the effect of neural excitation on DAF-16 in worms.

Discussion

We have shown that extended longevity and cognitive preservation in humans is associated with coordinate downregulation of genes that mediate excitatory neurotransmission. In the model system C. elegans, an increase in the activity of excitatory ASH neurons is a normal aspect of ageing. Global inhibition of neural excitation, or inhibition of specific excitatory neuronal populations—particularly glutamatergic or cholinergic neurons—resulted in robust extension of lifespan. These findings are consistent with previous studies, which showed that the anticonvulsants ethosuximide and valproic acid can extend lifespan in C. elegans. Moreover, we found that lifespan was dynamically regulated by the excitatory–inhibitory balance of neural circuits. Thus, an imbalance between neural excitation and inhibition might degrade neural function and contribute to the ageing process.

Our findings suggest that REST and the C. elegans orthologues SPR-3 and SPR-4 regulate ageing by acting as transcriptional repressors of synaptic genes and thereby reducing neural activity. Ageing conditional REST-deficient mice exhibit increased cortical neural activity and hyperexcitability. This is consistent with previous studies in neuronal cell culture, which suggested that REST maintains neuronal network homeostasis by buffering changes in neural excitation.

It is intriguing that REST and neural activity converge with insulin–IGF signalling to regulate the activity of forkhead transcription factors that play pivotal roles in lifespan regulation. The activation of daf-16 by REST orthologues in worms and FOXO1 by REST in humans might be a mechanism for integration of neural activity with metabolism.

We suggest that activation of REST and reduction of excitatory neural activity could be an approach to slowing ageing in humans.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41586-019-1647-8.
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METHODS
Brain sample procurement and description. Postmortem human brain material was procured in accordance with institutional guidelines and was approved by the Harvard Medical School Institutional Review Board. Tissue samples were procured from the Rush University Medical Center and the Brigham and Women’s Hospital. Tissue samples (both paraffin-embedded and frozen) from Rush University Medical Center were derived from participants in the Religious Order Study (ROS) and Rush Memory and Ageing Project (MAP) (together referred to in this RDS/MAP) at the Rush Alzheimer’s Disease Center; these are longitudinal, clinical–pathologic studies of ageing, cognitive decline and Alzheimer’s disease.39,40. Study participants agreed to comprehensive annual clinical and neuropsychological evaluation and to brain donation at death. Twenty-one cognitive function tests were used for the present study; including a summary score of all 17 tests used as a measure of global cognition, and separate measures of episodic, semantic, and working memory, perceptual speed, and visuospatial ability. The follow-up rate exceeds 95% and the autopsy rate exceeds 90%. All individuals who underwent autopsy cases were subject to a uniform structured neuropathological evaluation of Alzheimer’s disease, including assignment of Braak (measure of number and distribution of neurofibrillary tangles), CERAD (Aβ plaque pathology), and NIA-Reagan (composite measure of neurofibrillary tangles and amyloid plaques) scores (https://www.radc.rush.edu/docs/var/detail.htm?category=Pathology&subcategory=Alzheimer%27s&%20disease&variable=cerads). Paraffin-embedded brain samples were also obtained from the Brigham and Women’s Hospital. These samples included tissue from young adults without neurological abnormalities.

Immunofluorescence analysis of human brain. Immunofluorescence analysis of the prefrontal cortex (Brodmann area 9) was carried out using paraffin-embedded brain sections. Paraffin-embedded tissue sections were first deparaffinized in xylene, then rehydrated with decreasing concentrations of ethanol and placed in water. Sections then underwent antigen retrieval using the Diva decoyser (BioCare, USA). They were then washed and blocked with 2% BSA and 0.1% Triton X-100 in PBS for 1 h at room temperature. Primary antibodies were diluted in 2% BSA and 0.1% Triton in PBS. Following overnight incubation at 4°C, sections were washed three times with PBS. Secondary antibodies, diluted in 2% BSA and 0.1% Triton in PBS were either biotin-conjugated (1:200, Vector Labs, USA) or coupled to Alexa fluorophores (1:300, Invitrogen). Sections were incubated with 1% Sudan Black in 80% ethanol for 10 min at room temperature to suppress lipofuscin autofluorescence. Following washes in PBS, sections were mounted and imaged using confocal microscopy. The following antibodies were used for immunolabelling: (i) a rabbit polyclonal IgG that recognizes a region between residues 1050 and the C terminus (residue 1097) of REST (Bethyl laboratories, HIC-00141); (ii) a goat polyclonal IgG that recognizes the C-terminal region of FOXO1a (LSBiO B415, discontinued, replaced with LSBio 1322). To quantify immunofluorescence, images that were randomly acquired in selected brain regions were analysed using the MetaMorph software (Molecular Devices). Antigen expression was quantified within each neuron (such as the nucleus) were selected using the average signal intensity measured. Values were corrected by subtracting the average slide background intensity (measured outside cells). The investigator was blinded to sample origin or diagnosis.

To assess the relationship between the levels of nuclear REST protein and FOXO1, we performed confocal immunofluorescence with triple-labelling for REST, MAP2 and FOXO1. Multiple 40× pictures were acquired (at various locations) within the prefrontal cortex displaying pyramidal neurons using an Olympus Fluoview Confocal Microscope. For cases displaying a majority of pyramidal neurons with very high (or very low) nuclear REST levels, fields were also included that displayed lower (or higher, respectively) REST levels, to test for potential correlations between REST and FOXO1. Antigen-expressing areas within neuronal nuclei were selected using the Metamorph image analysis system and the average signal intensity measured. Values were corrected by subtracting the average slide background intensity (measured outside cells). Between 70 and 115 pyramidal neurons were quantified for each case.

Conditional REST knockout mice. Animal housing and experimental procedures were approved by the Institutional Animal Care and Use Committee of Harvard Medical School. Mice carrying floxed alleles of REST flanking exon 2 were described previously.33,35. These mice were crossed to Nestin-Cre transgenic mice (Jackson laboratory; strain 003771) to achieve conditional inactivation of REST in the nervous system. The Nest-Cre transgene is in the C57BL/6 background, and the RestΔ3-8 alleles were in a hybrid C57BL/6 and 129Sv/Ev background. The resulting Nestin-Cre/RestΔ3-8 conditional knockout mice (hybrid C57BL/6 and 129Sv/Ev background), referred to as Restcko, were born at expected Mendelian ratios, were viable and fertile, and did not display any visible alterations. The control groups included RestΔ3-8 and Nest-Cre mice. Mouse genotyping by PCR was performed using primers specific for the REST flanking exon 2: re08 (5’-CTAGGCGACTTGGCCAGGT-3’), re09 (5’-GTGATGCGCGCTTCTGGAAGG-3’), and re11 (5’-GGACACCTTTTAATCCAGCTTC-3’); this allowed the identification of wild-type (220 bp), floxed (264 bp) or recombinant (375 bp) Rest alleles. The experimental groups included both male and female mice in equal proportions. The Restcko and RestΔ3-8 control groups were composed of littermate mice on the same genetic background. Mice were identified by eartag numbers, and were randomly selected for PET–CT and EEG experiments, as well as for histological processing (perfusion, brain dissection, and so on).

Immunofluorescence analysis of mouse brain. Mice were anaesthetized with isoflurane, carbon dioxide and then perfused with cold PBS buffer for 20 min. Brains were rapidly removed and placed in 4% PFA overnight at 4°C. They were then processed for paraffin embedding, according to standard procedures. The investigator was blind to the genotype. To assess FOXO1 nuclear expression in cortical neurons, coronal brain sections from wild-type and Restcko mice (aged 9 or 18 months) were immunolabelled with FOXO1 and MAP2 antibodies. Cortical neurons (MAP2+ ) were identified and the mean FOXO1 fluorescence intensity in each nucleus was measured using Metamorph software. Between 50 and 100 neuronal nuclei were assessed for FOXO1 expression, and the mean FOXO1 nuclear expression was derived for each animal. To confirm REST deficiency (Extended Data Fig. 5e), Restcko mouse cortical sections were labelled with an anti-REST antibody provided by the Hsieh laboratory (Supplementary Table 20).

PET–CT of Restcko mice. Mice were anaesthetized with 3% isoflurane (Baxter Medical) and medical grade oxygen at a rate of 1 l/min. A CT scout scan was done first, followed by a CT scan and a dynamic PET scan. Each mouse received the same dose per gram of body weight (1.75 Ci/g) of [18F]fluorodeoxyglucose (FDG) tracer solution by tail vein injection, followed by a 0.1 ml saline flush. Dynamic PET imaging for each mouse was immediately performed for 1 h (in vivo PET) and then for a further 3 h (ex vivo PET). The spatial resolution of the PET scan was 1.6 mm at the centre of the field of view. The data were acquired in 3D mode at the energy window of 250–700 keV, which yields 4% count sensitivity. For each time point, five or six 3D volumes, spanning cortical and subcortical regions, were selected in the centre of the brain, with volumes of 0.2 cm3 each, and used for quantification using the eXplore Vista software. The averages of these regions were used as the SUV for the animal.

Cell culture. Primary cortical neuronal cultures, derived from E16.5 wild-type C57BL/6 fetuses, were plated in 10% serum-containing neuronal culture medium (neurobasal medium containing B27 supplements, penicillin, streptomycin, and GlutaMax) on either coverslips or culture dishes that were pre-coated with poly-l-ornithine (Sigma p4957). The medium was changed 4 h after initial plating to serum-free neuronal culture medium, and then a half-medium change was performed every three days.

Electroencephalograph of Restcko mice. Electroencephalograph (EEG) telemetry unit implantation. Mice were implanted with wireless telemetry units (PhysioTel ETA-F10; Data Sciences International, DSI, St. Paul, MN) under appropriate sterile techniques per laboratory protocol as previously described.33,35,36 Electroencephalogram (EEG) analysis. EEG experiments, as well as for histological processing (perfusion, brain dissection, and so on).
Immunocytochemical analysis of cultured cells. Embryonic mouse cortical neuronal cultures as described above were plated on poly-l-ornithine-coated cover slips. Stocks of NBQX, APV (Tocris Bioscience Cat. No. 0190, 0106, respectively), and kynurenic acid sodium salt (Abcam, ab146693) were added to neurobasal medium and used in a half-medium change for a final concentration of 5 μM kynurenic acid, 50 μM APV or 2 μM NBQX at day 10 of culture. After 24 h, the culture medium was aspirated and cells were fixed by incubation with 4% (v/v) paraformaldehyde in PBS for 20 min at room temperature, and permeabilized with 0.2% Triton X-100 in PBS for 15 min at room temperature. After a wash in PBS, cells were blocked with 4% BSA in PBS overnight at 4°C. Primary antibodies were diluted to the appropriate concentration in 4% BSA, and incubated with the cells overnight at 4°C. Cells were then washed three times in PBST (PBS with 0.05% Triton) for 10 min each, before we added fluorescent-conjugated secondary antibodies for 2 h at room temperature. Fluorophore-labelled cells were then washed in PBST (3 × 10 min) and mounted using Prolong Gold mounting medium with DAPI and anti-fade reagent (Invitrogen). The primary antibodies used in Fig. 5d are rabbit anti-FOXO1 (Cell Signaling 2880) and chicken anti-MAP2 (Sigma–Millipore, AB5392). Nuclei were identified by DAPI labelling, and the cellular distribution of FOXO1 labelling was quantified using Metamorph software.

Cell identification parameters were optimized for scoring neuronal cultures, and then the same image analysis macro was applied to all images to generate average FOXO1 cellular intensities in MAP2-positive neurons.

C. elegans strains. The N2 Bristol strain was used as the wild-type background for these studies. C. elegans strains were cultured at 20 °C under standard conditions as described. The following mutations and chromosome rearrangements were used: LGII: mec-4[+]; pr-5[+], was mildly anaesthetized with 0.01% tetramizole and outcrossed to the indicated worm strains. For transgenic experiments, 450 worms per genotype were grown on 100 μL FUDR-containing plates (starting at L4) and collected in M9, either on day 2 or day 10 of adulthood. Worms were washed once in M9, pelleted by centrifugation, resuspended in 200 μL Trizol, vortexed for 2 min and flash frozen in liquid nitrogen. Worms were then freeze-cracked by thawing in a 37 °C water bath and re-freezing in liquid nitrogen. This was repeated two more times. After the final thaw, 100 μL Trizol was added and the tubes were maintained at room temperature for 5 min. RNA was then extracted with 140 μL chloroform, precipitated with an equal volume of 70% ethanol and transferred to an RNasey spin column (Qiagen) and purified. Quantitative RT–PCR was performed directly from isolated RNA, using 1 ng of RNA and the Qiangen One step qPCR mix. All reactions were performed in triplicate. The ddCt method was used to analyse qRT–PCR data, and ddCt values were used for statistical analysis.

C. elegans motility assay. Worms were treated for 24 h with the indicated drugs, and then 150 worms were transferred to 1.5 mL liquid nematode growth medium (NGM; 1 mM MgCl₂, 1 mM CaCl₂, 200 mM KH₂PO₄, 50 mM NaCl) and washed once to remove bacterial clumps. Worms were transferred in 100-μL volumes to a 96 u-shaped-well plate, and assayed in the nemateMet wMicrotracker (https://nematome.com/products/phenotyping-products/wmicrotracker/) according to the manufacturer’s instructions.

C. elegans lifespan determination and stress treatments. Lifespan and ageing experiments were performed at 20 °C and on fluoredoxine (FUDR) unless otherwise noted. For each genotype, 20–35 day 1 worms were transferred to NGM plates containing 100 μg/mL FUDR. Worms were scored for viability every day or every other day, and transferred to fresh plates between day 10 and day 14. For daf-2 mutant worms, worms were moved again at day 20–24. For histamine and ivrvermectin experiments, where the same plates were used for the entirety of the lifespan, a wetted towel was placed in the container with the plates to mitigate evaporative loss of water from the plates. For lifespan experiments in which untreated worms were transferred to the treatment group (for example, day 8 histamine treatment), control worms with or without treatment were also transferred to fresh plates of the same type to control for any lifespan effects resulting from plate transfer. The presence or absence of FUDR is indicated for each experiment in Supplementary Table 22.

For all lifespans and stress resistance experiments, worms that burst (interior leaking out through the vulva) or begged (interior hatching of progeny) were discarded. Worms that did not use lifespan analysis, along with animals that crawled off the plate during the course of the assay. Plates with mould or other contamination were discarded.

Quantification of DAF-16::GFP fluorescence. For quantification of GFP fluorescence in fixed animals, 25–30 worms were transferred to an Eppendorf tube containing M9 buffer, washed once in M9 and pelleted. The supernatant was removed and the pellet was frozen in liquid nitrogen. The day before imaging, worm pellets were thawed in PBS with 4% paraformaldehyde (ThermoFisher) and fixed for 30 min at room temperature while rocking. The FFA was removed by washing twice with PBS, with 0.025% Triton (PBST, to prevent sticking), and the worms were stained with DAPI for 10 min at room temperature. The DAPI was removed with a final wash in PBST, and the pellet was resuspended in 15 μL ProLong Gold mounting medium (Life Technologies). The worms were transferred in 12.5 μL mounting medium to a slide, and gently placed under a coverslip, which was then sealed with clear nail polish (Electron Microscopy Sciences).

Slides were imaged on an Olympus Fluoview 1000 confocal microscope, using manufacturer settings for GFP and DAPI (488 nm and 405 nm laser, respectively) within 2 weeks of mounting, and all slides compared with each other were imaged on the same day, in the same imaging session. Worms were imaged via a 40 × objective with a 2 × digital zoom. For every worm, Z-stack image series were taken at 2-μm step sizes (roughly 13–15 images per series). To quantify nuclear GFP levels, these Z-stacks were opened in ImageJ (NIH) and flattened using a maximum intensity Z-stack projection. For pharyngeal nuclei, three regions of interest (ROI) were selected on the basis of DAPI staining of nuclei, on either side of the pharynx and behind it, encompassing the bulk of the nuclei in that area. These were then used as the ROIs within which to measure GFP fluorescence, using the ImageJ intensity measuring tool. Average scores from the ROIs were computed for each condition. Raw data files were uploaded to the Database for Integrateomics (DIF) and Extended Data Fig. 9a (right), all nuclei located in the middle three μm steps of the Z-stack were individually selected using the same ROI and intensity measuring tools. These nuclei were then used to calculate an average score per worm.
GA4XAP imaging in *C. elegans*. Data acquisition. GA4XAP imaging was performed in lines bearing kyIs602 [sra-6:GA4XAP3.0, 75 ng/µl + unc-122:GFP, 10 ng/µl], which expresses predominately in the ASH neurons. Worms were removed from plates and mounted on 7.5% agarose pads in liquid NGM (1 mM MgCl2, 1 mM CaCl2, 200 mM KH2PO4, 30 mM NaCl), mixed 1:1 with 0.05-µm polystyrene beads (Polysciences, Inc., cat. 08691) as described (http://sbg.worm- book.org/2009/12/01/agarose-immobilization-of-c-elegans). A coverslip was very gently piled and worms were imaged for a maximum of 30 min after mounting. Videos lasting 144 s (240 frames, –0.6 frames per second) were recorded on the confocal microscope described above (FV1000), with the confocal aperture widened to 250 µm to allow lower excitation intensity and mitigate z-drift. Worms were imaged with factory GFP settings and the 488-nm laser set to 2%. Videos were opened and played in Image J, and excitation events were scored manually for Fig. 4a. Scoring was performed blinded to genotype.

Analysis of GA4XAP intensity changes. For analyses of ∆F/F0 maximum intensity, videos were analysed using a custom MATLAB script, which automatically registered and segmented the ASH neuron and recorded GA4XAP fluorescence intensity for each frame. Videos of tracked neurons were manually reviewed and neurons that were poorly tracked were excluded from analysis. To normalize the fluorescence intensity per worm we used ∆F/F3, calculated as (F3 – F0)/F0 where F3 is the fluorescence intensity at time t and F0 represents an estimate of the baseline fluorescence, calculated as the 0.2 quantile fluorescence per worm. ∆F/F0 maximum intensity was the maximum ∆F/F0 over the entire recording (240 frames). For analysis of nemadipine-treated worms and for worms expressing the HisCl channel (and their controls), ROIs were hand-drawn because the tracking program failed, owing either to low signal intensity (nemadipine-treated worms) or the inability to distinguish a confounding GFP signal (HISCl:GFP worms). Worms were used in multiple GA4XAP analyses.

*C. elegans* cloning and genotyping. The SPR-4:GFP fusion fosmid was used in bombardment (Extended Data Fig. 6a, b; clone: 3167840880351681 C09) was provided by the Transgene consortium49. All primers used for genotyping can be found in Supplementary Table 2.

RNA library preparation and sequencing. Human RNA-seq. Details of RNA-seq sample preparation kits from 500 ng of purified total RNA according to the manufacturer's protocol. The finished dsDNA libraries were quantified as gene counts using FeatureCounts v1.5.151 with options -C -p -B -s

RNA library preparation and sequencing. Human RNA-seq. Details of RNA-seq sample preparation kits from 500 ng of purified total RNA according to the manufacturer's protocol. The finished dsDNA libraries were quantified as gene counts using FeatureCounts v1.5.151 with options -C -p -B -s
Throughout the paper, ‘individuals with extended longevity’ refers to individuals in the oldestold groups defined above.

C. elegans day 2 and day 10. There were four genotypes of worms: daf-2, daf-2; spr-4; spr-3, N2, and spr-4; spr-3, and each genotype had three biological replicates. Each genotype was considered as a separate group for analysis.

**Gene expression normalization and covariate adjustment.** ROansom cohort. Gene counts were input to edgeR. Genes were deemed to be expressed if $\geq 10$ individuals in a combined youngold and oldestold group had $1 \ CPM$. Genes not satisfying these criteria were removed, keeping the original library sizes. These were expressed genes used during each iteration of outlier analysis. After outlier analysis, this filtering retained 18,511 expressed genes out of 58,051 annotated genes for analyses involving the ROansom cohort. Counts were then normalized using the TMM method in edgeR. Finally, log(CPM) values were calculated for analyses other than differential expression.

To adjust gene expression for covariates we fit the linear regression model for each gene separately using lin(m) in R: gene expression $- g$roup $+$ covariates where gene expression is log(CPM), and using the group and covariates for ROansom. For ROansom, group was a factor with four levels: youngold, middleold, oldestold, and cognitivedecline, with cognitivedecline as the reference level. The covariates were sex (factor, two levels), RIN (continuous), RIN$^{+}$ (continuous), PMI (continuous), and sequencing batch (factor, eight levels). The final normalized and adjusted gene expression values were derived from adding the regression residuals to the estimated effect of the group level to preserve the effect of the group on expression. These normalized and adjusted gene expression values were used to perform gene–gene regression analysis and gene–gene group regression analysis, and to visualize differentially expressed genes in heat maps.

**CommonMind Consortium cohort.** Gene counts were input to edgeR. Genes were deemed expressed if $\geq 10$ individuals in a combined youngold and oldestold group had $\geq 1 \ CPM$. Genes that did not satisfy these criteria were removed, maintaining the original library sizes. These were expressed genes used during each iteration of outlier analysis. After outlier analysis, this filtering retained 19,453 expressed genes out of 56,632 annotated genes for analyses involving the CMC cohort. Counts were then normalized using the TMM method in edgeR. Finally, log(CPM) values were calculated for analyses other than differential expression. To adjust gene expression for covariates, we fit the linear regression model for each gene separately using lin(m) in R: gene expression $- g$roup $+$ covariates where gene expression is log(CPM), and using the group and covariates for CMC. For CMC, group was a factor with five levels: young, youngold, middleold, oldestold, and neuropsychiatric illness, with neuropsychiatric illness as the reference level. The covariates used were selected from those used in the original publication: sex (factor, two levels), RIN (continuous), RIN$^{+}$ (continuous), PMI (continuous), clustered batch (factor, nine levels), and institute (factor, three levels). The final normalized and adjusted gene expression values were derived by adding the regression residuals to the estimated effect of the group level to preserve the effect of the group on expression. These normalized and adjusted gene expression values were used to perform gene–gene regression analysis and gene–gene group regression analysis, and to visualize gene expression.

**Gibbs cohort.** Gene expression data measured on the Illumina humanRef-8 v2.0 expression beadchip platform were downloaded from NCBI GEO GSE15745. Raw intensity values for each probe were transformed using the rank invariant normalization method by the authors$^{1,2}$, and then log, transformed for analysis. Probes were deemed expressed if $\geq 10$ individuals in a combined youngold and oldestold group had $P < 0.01$. Unmapped probes according to updated GEO Platform (GPL) annotation were removed. These were expressed probes used during each iteration of outlier analysis. After outlier analysis, this filtering retained 13,239 expressed probes out of 22,184 probes for analyses involving the Gibbs cohort.

To adjust gene expression for covariates we fit the linear regression model for each gene separately using lin(m) in R: probe expression $- g$roup $+$ covariates where probe expression is log, expression, and using the group and covariates for Gibbs. For Gibbs, group was a factor with four levels: young, youngold, middleold, and oldestold, with young as the reference level. The covariates were sex (factor, two levels), PMI (continuous), and prep hybridization batch (factor, seven levels). The final normalized and adjusted probe expression values were derived by adding the regression residuals to the estimated effect of the group level to preserve the effect of the group on expression. These normalized and adjusted probe expression values were used to visualize probe expression.

C. elegans day 2 and day 10. Gene counts were input to edgeR. Genes were deemed expressed if $\geq 3$ samples had $\geq 1 \ CPM$. Genes not satisfying these criteria were removed, keeping the original library sizes. This filtering retained 12,981 expressed genes out of 46,739 annotated genes for analyses involving C. elegans day 2 and 15,154 expressed genes involving C. elegans day 10. Counts were then normalized using the TMM method in edgeR. Finally, log(CPM) values were calculated for analyses other than differential expression. For day 2, covariate adjustment was not necessary because there were no other covariates beyond the group variable. For day 10 we did not include a batch covariate because there was no observed batch effect on a principal components plot, and results were similar if a batch covariate was included and the two dropped technical repeat sequencings were used instead of their original sequenced reads (data not shown). Thus, covariate adjustment was not necessary because there were no other covariates beyond the group variable. These normalized gene expression values were used to visualize gene expression.

**Differential expression analysis.** ROansom cohort. Differential expression analysis between the oldestold and youngold groups with covariate adjustment using the covariates listed above for ROansom was performed for expressed genes using edgeR (estimateDisp, glmFit, and glmLRT with default arguments) in R. All 592 samples were included to increase statistical power during covariate modelling. Genes were considered differentially expressed if FDR $\leq 0.05$ and the absolute value of the fold change was $\geq 1.2$.

**Gibbs cohort.** Differential expression analysis between the oldestold and youngold groups with covariate adjustment using the covariates listed above for Gibbs was performed for expressed probes using linear regression models in limma (lmFit and eBases with default arguments) in R. All 144 samples were included to increase statistical power during covariate modelling. Probes were considered differentially expressed if FDR $\leq 0.05$. Genes and set enrichment analysis. Gene annotation and genes used for functional gene classification into biological process (BP), molecular function (MF), and cellular component (CC) were from the GRCh38.p7 database downloaded from Ensembl Biomart (https://www.ensembl.org/biomart/martview/) on November 2, 2016.

**Synaptic transmission genes.** Genes that had direct or indirect annotation for the GO biological process ‘Chemical synaptic transmission’ (GO: 0007268).

To identify REST target genes, the REST REI motif position–specific weight matrix (PSWM) was trained using the genome sequence GRCh38 to predict REST binding sites. A gene was defined to be a REST target if it had an REI motif with $P < 1 \times 10^{-7}$ that was $\geq \pm 10 \ kb$ from the transcription start site of any transcript of the gene in the Ensembl GRCh38.86 gene models. This procedure identified 2,632 REST target genes before filtering for expressed genes in each cohort during gene set enrichment analysis.

**ENCODE ChIP–seq transcription factor target gene sets.** Gene sets were from the ENCODE_TF_ChIP_seq_2015 database downloaded from http://www.encodeproject.org. Cell type analysis gene sets were derived from a transcriptome database of the major cell classes of the mouse cerebral cortex $^{57}$ using data from https://web.stanford.edu/group/barres_lab/brain_rnaseq.html. To select cell marker genes, first we calculated the fold expression of each gene in each cell type by dividing the fragments per kilobase of transcript per million mapped reads (FPKM) expression in that cell type by the mean of the FPKM expression in the other six cell types. For each cell type we selected as cell marker genes those that had at least tenfold higher expression in that cell type and that had one-to-one human–mouse homologues. Homologues were downloaded from http://www.informatics.jax.org/downloads/reports/HOM_MouseHumanSequence.rpt on November 16, 2016.

**Cell type analysis gene sets.** Gene sets were derived from a transcriptome database of the major cell classes of the mouse cerebral cortex $^{57}$ using data from https://web.stanford.edu/group/barres_lab/brain_rnaseq.html. To select cell marker genes, first we calculated the fold expression of each gene in each cell type by dividing the fragments per kilobase of transcript per million mapped reads (FPKM) expression in that cell type by the mean of the FPKM expression in the other six cell types. For each cell type we selected as cell marker genes those that had at least tenfold higher expression in that cell type and that had one-to-one human–mouse homologues. Homologues were downloaded from http://www.informatics.jax.org/downloads/reports/HOM_MouseHumanSequence.rpt on November 16, 2016.

**DAF-16 class I (upregulated) genes.** Genes were derived using genes with FDR $< 0.01$ and class $== \uparrow$ from Supplementary Table 1 of Tepper et al. $^{58}$.

For gene set enrichment analysis of differentially expressed genes, we retained only genes in each gene set that were expressed in that differential expression analysis. Gene set enrichment analysis was performed separately for upregulated genes and non–upregulated genes, downregulated genes and non–downregulated genes, and differentially regulated genes and non–differentially regulated genes. For gene set enrichment analysis of GO biological process gene sets, gene set enrichment was determined using the topGO R package $^{59}$ using the classic algorithm and Fisher’s statistic. Gene set enrichment for REST target genes, ENCODE ChIP–seq transcription factor target gene sets, and cell type analysis gene sets was performed using the hypergeometric distribution.
Gene sets with fewer than five genes after filtering for expressed genes were removed before the gene set enrichment false discovery rates were calculated. To calculate the statistics for overlap of dag2-2 upregulated genes, spr-4(spr-3)dag2-2 upregulated genes, spr-4(spr-3)dag2-2 downregulated genes, and dag-16 class I genes for C. elegans day 10 we used the SuperExactTest version 1.0.0 R package. 

For meta-analysis of GO terms and ENCODE ChiP-seq transcription factor target gene set enrichment from the ROSMAP, CMC, and Gibbs cohorts, we used Stouffer’s method with weights for combining P values implemented in the sumx function in the metap R package. P values equal to 1 were replaced with 0.999999 to comply with the requirement that 0 < P < 1. For study weights we used the square root of the total number of individuals in the combined youngold and oldstold group for each study (ROSMAP, n=117; CMC, n=155; Gibbs, n=37) 

Hierarchical clustering to select age group cutoffs. Normalized and adjusted gene expression values were derived as described in the section Gene expression normalization and covariance adjustment, with the following changes. First, the group variable was a factor with levels c0x1, c0x2, c0x3, c0x4, c0x5, and c0x6 (ROSMAP); control, schizophrenia, bipolar, affective (CMC); or not included (Gibbs). Next, genes were deemed expressed if ≥ 10 individuals in the cognitively normal group (ROSMAP, c0x1; CMC, control with age ≥ 60) had more than 1 CPM. Genes not satisfying these criteria were removed, keeping the original library sizes. For the Gibbs cohort, probes were deemed expressed if ≥ 10 individuals with age ≥ 55 had detection P < 0.01 and no samples had NA values. Probes not satisfying these criteria were removed. These were expressed genes used for analysis. This filtering retained: ROSMAP, 18,734 expressed genes; CMC, 19,615 expressed genes; and Gibbs, 13,411 expressed probes. For RNA-seq data, counts were then normalized using the TMM method in edgeR, and log(CPM) values were calculated for expression. Finally, age was included as a continuous covariate, and the effects of group and age were added back to the residuals. Then cognitively normal aged individuals were selected in each cohort (ROSMAP, c0x1; CMC, control with age ≥ 60; Gibbs, age ≥ 55). To determine age-associated genes, the expression of each expressed gene was correlated with the age of death of the individual using Spearman’s correlation. Genes with Spearman rank correlation FDR < 0.1 (ROSMAP, n=1,025 genes) or FDR < 0.05 (CMC, n=6,828 genes; Gibbs, n=203 probes) were age-associated genes. Permuting sample ages before calculating the Spearman correlations gave no genes that passed the FDR threshold. Genes were then normalized to have mean 0 and s.d. 1 across individuals, and genes with normalized expression ≥ 3 or ≤ -3 were set to 3 or -3, respectively. The Pearson correlation coefficient between individuals was then calculated using only age-associated genes. 

Agglomerative hierarchical clustering using the Euclidean distance metric and average linkage criterion was performed on the matrix of pair-wise Pearson correlations to relations to cluster individuals. The resulting tree was cut into three groups, and the distance of the root of each cluster with the age of death of individuals of each group was analysed. Using the ROSMAP cohort, for the cluster with the smallest median age, with at least 25 individuals in the youngold group and at least 75 individuals in oldstold group. On the basis of the top scoring age cutoffs in the ROSMAP cohort, we selected y0 = 80 and o0 = 85. Thus, the age cutoffs for defining the youngold, middleold, and oldstold groups used for differential expression in the ROSMAP, CMC, and Gibbs cohorts were selected to be <80, ≥80 and <85, and ≥85, respectively. 

Statistical analysis and data representation. Statistical analysis was performed using R. Statistical tests used are noted in the figure legends or in the relevant Methods section. Throughout the paper, all tests are two-sided and unpaired unless stated otherwise. A significance level of 0.05 was used to reject the null hypothesis unless stated otherwise. 

Box plots throughout the paper show the median, lower and upper hinges (first and third quartiles), upper whisker (hinge to the largest value no further than 1.5 × IQR from the hinge), lower whisker (hinge to the smallest value at most 1.5 × IQR from the hinge), and outlying points beyond the whiskers. Additionally, all points are plotted on top of the box plot and randomly jittered horizontally. A t-test was used for parametric comparisons between two groups with normally distributed data, and used t.test() for groups with equal variance, Student’s t-test with unequal variances used t.test() with argument var.equal = TRUE; otherwise Welch’s t-test was used (argument var.equal = FALSE). ANOVA was used for parametric comparisons between more than two groups and used aov(). The Mann–Whitney U-test was used for nonparametric comparisons between two groups without knowledge of their distribution and used wilcox.test() with arguments exact = TRUE, correct = FALSE. Levene’s test was used to investigate the homogeneity of variance across groups and used leveneTest() from the car R package. Q–Q plots and the Shapiro–Wilk test were used to assess normality and used shapiro.test(). The Bonferroni outlier test was used to assess outliers and used outlierTest() from the car R package. The log-rank test was used to compare the survival distributions of two groups. Survival statistics were calculated using survfit() with argument survival = TRUE and used coxph() and survdiff(). To determine whether there was a linear relationship between two variables we fit a linear regression model and tested the null hypothesis that the slope of the regression line is 0 using a two-sided t-test using lm(). P values were corrected for multiple comparisons where noted using p.adjust() with argument method = “fdr”, “holm”, or “bonferroni” for false discovery rate, Holm’s method, or Bonferroni correction, respectively. The GCAMP imaging data indicated that nonparametric analyses were most appropriate and thus were used for all GCAMP analyses. In Fig. 2e, the presence of candidate outliers and/or potential non-normality for time points 1–12.5 min suggested that a Mann–Whitney U-test was more appropriate although a t-test produced similar conclusions. Meta-analysis of lifespan experiments that performed pairwise comparisons among more than two groups (Extended Data Fig. 8a right, Extended Data Fig. 8c) were pairwise Student’s t-tests implemented in pairwise.t.test() with argument pool.sd = TRUE to calculate a common s.d. used for all groups and comparisons.

Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

Data availability

Data from the ROSMAP cohort are available under controlled use conditions set by human privacy regulations. To access the data, a data use agreement is needed. This registration is in place solely to ensure the anonymity of the ROSMAP study participants. Data can be requested only from the Rush Alzheimer’s Disease Center Resource Sharing Hub at http://www.radc.rush.edu/. C. elegans RNA-seq data are available in the Gene Expression Omnibus (GEO) under accession number GSE123146.

Code availability

All code used in the analysis of data presented in this manuscript is available upon request.

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Competing interests G.M.C. is a cofounder and senior advisor for GC Therapeutics, Inc, which uses transcription factors for therapeutics. The other authors declare no competing interests.

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Extended Data Fig. 1 | Partitioning of the ageing human population for analysis of gene expression in the brain. a–c, Adjusted gene expression profiles of age-associated genes were compared between cognitively normal aged individuals to derive a matrix of Pearson correlation coefficients that indicate the degree of similarity between any two cases in the ROSMAP (a, dorsolateral prefrontal cortex, n = 150 individuals), CMC (b, dorsolateral prefrontal cortex, n = 174 individuals) and Gibbs (c, frontal cortex, n = 40 individuals) cohorts. d–f, Most significantly enriched GO terms for upregulated genes in the cortex of cognitively normal individuals who lived to be ≥85 years old relative to individuals who lived to be ≤80 years old in the ROSMAP (d, n = 117 individuals), CMC (e, n = 155 individuals), and Gibbs (f, n = 37 individuals) cohorts. P values were calculated using Fisher’s exact test (see Methods). g, Meta-analysis of GO term enrichment for downregulated genes. Shown are selected GO terms related to excitatory and inhibitory synaptic transmission. The individual cohort enrichment P values were combined using Stouffer’s method (see Methods). NS, not significant (FDR > 0.1).
Extended Data Fig. 2  | Ivermectin and nemadipine extend lifespan without interfering with worm motility. a, Worms were transferred at day 8 to either standard NGM plates or plates containing ivermectin (Ive, 1 pg ml⁻¹) or nemadipine (2 μM). Shown is a representative curve of an experiment repeated twice. Nemadipine versus wild-type, \( P = 3.2 \times 10^{-4} \); ivermectin versus wild-type, \( P = 2.2 \times 10^{-7} \) by log-rank test. Nemadipine, \( n = 81 \); ivermectin, \( n = 82 \); wild-type, \( n = 76 \). b, Day 2 worms treated with nemadipine or ivermectin for 24 h were transferred to liquid culture and thrashing rate was assessed using the Nemametrix wMicrotracker (see Methods). Shown are mean motility scores for the first 60 min ± s.e.m. Untreated, \( n = 17 \) wells; ivermectin, \( n = 17 \) wells; nemadipine, \( n = 16 \) wells. Each well contained about 10 worms. **\( P = 1.7 \times 10^{-4} \) versus untreated, Mann–Whitney U-test with multiple testing correction by Holm’s method. Results are representative of an experiment replicated twice.
Extended Data Fig. 3 | Repression of multiple neurotransmitter systems extends lifespan in *C. elegans*. a–h, *C. elegans* lines expressing the transgenic HisCl1 channel in the indicated neuronal populations were treated with 10 mM histamine (His+) starting at adult day 1 (a, c, e, g) or day 8 (b, d, f, h) and compared to untreated controls (His−). *P* values calculated by log-rank test. See Supplementary Table 22 for individual *n* values and statistics. i, Mean lifespan extension ± s.e.m. for worms treated with histamine at days 1 or 8 relative to untreated controls for at least three independent replicates. *P* < 0.05, ** *P* < 0.01 by Student’s *t*-test. HisCl1 was driven using the GAL4SK:VP64 system for the GABAergic (GABA), glutamatergic (GLUT) and cholinergic systems, using *unc-47*, *cat-4*, and *unc-17* drivers, respectively (see Supplementary Table 19). j, Reduced ASH neuron excitation following inhibition of GABA activity at day 1 but not day 8. Shown is normalized maximum GCaMP fluorescence in day 1 and 8 *unc-47*HisCl1 worms that were treated with 10 mM histamine (His+) on the indicated day, or untreated controls (His−). Day 1 His−, *n* = 18 worms; day 1 His(+), *n* = 19 worms; day 8 His−, *n* = 23 worms; day 8 His(+), *n* = 20 worms. *P* = 1.1 × 10−3 by Mann–Whitney U-test.
Extended Data Fig. 4 | Neural excitation, neuropeptide signalling and lifespan in *C. elegans*. a, Increased excitation of ASH neurons following RNAi against the GABA vesicular transporter *unc-47*. GCaMP imaging was performed on worms with enhanced neuronal RNAi (see Fig. 3 legend and Methods) for *unc-47* (*n* = 37) or controls (*n* = 43) at day 2. **P = 6.8 × 10^-3 by Mann–Whitney U-test.** b, RNAi of *unc-47* reduces lifespan. Worms with enhanced neuronal RNAi were treated with *unc-47* (*n* = 31) or control RNAi (*n* = 84). Shown is a representative lifespan analysis replicated three times. **P = 1.3 × 10^-6 by log-rank test.** c, Reduction in synaptic neurotransmission or neuropeptide signalling extends lifespan in *C. elegans*. Mutations in genes affecting glutamatergic neurotransmission (*eat-4*), presynaptic function (*unc-13*) and neuropeptide signalling (*egl-3*) produce comparable lifespan extensions. WT, *n* = 57; *eat-4*(*nj2*), *n* = 54, P ≤ 2.2 × 10^-16; *unc-13*(*e51*), *n* = 92, P = 3.6 × 10^-14; *egl-3*(*gk238*), *n* = 35, P = 8.3 × 10^-11 by log-rank test. Curves are representative of two independent replicates. d, Extension of lifespan by *egl-3* RNAi in worms with enhanced neuronal RNAi. Shown are lifespan curves representative of two independent replicates. *egl-3* RNAi (*n* = 47 worms); empty vector (*n* = 84 worms). **P = 3.5 × 10^-11 by log-rank test.**
Extended Data Fig. 5 | Gene regulation and neural activity associated with REST and extended longevity. a, b, Expression of genes that are downregulated in individuals ≥85 years old versus ≤80 years old is inversely related to REST mRNA levels. Shown is linear regression analysis of normalized and adjusted REST mRNA levels and mean expression of all downregulated genes (a) and downregulated genes associated with the synaptic transmission GO term (b). Data are from the CMC cohort. Each point represents an individual case, n = 155 individuals. P values derived by t-test for the slope of the regression line. Note similarity to the data for the ROSMAP cohort in Fig. 2a, b. c, d, Stratification by age group. Analysis of the ROSMAP cohort (c, n = 117 individuals) and the CMC cohort (d, n = 155 individuals) as in Fig. 2a, but stratified by age group. P values derived by t-test for the slope of the regression line. e, Loss of REST expression in conditional Rest knockout mice. Representative images of the cortex (top) and hippocampus (bottom) from Rest+/− (control) and Nestin-Cre;Rest+/− (Rest−/−) mice. Immunolabelling was performed with the anti-mouse REST-14 antibody directed against the REST C-terminal domain (Supplementary Table 20). Scale bar, 40 μm. Image is representative of an experiment replicated four times. f, Survival of Rest−/− and control mice following administration of the seizure-inducing agent PTZ (40 mg kg−1x Rest−/− versus control by log-rank test. Control, n = 9; Rest−/−, n = 7.)
Extended Data Fig. 6 | Induction of spr-4 extends lifespan and suppresses neural excitation in *C. elegans*. a, spr-4 mRNA levels in worms expressing a stably integrated dCas9::VP64 transgene in the presence (sgRNA(+)) or absence (sgRNA(−)) of four different sgRNAs targeting the spr-4 promoter. Transcript levels were determined by qRT–PCR and normalized to sgRNA(−) controls. Mean ± s.e.m., n = 3. Primer A, *P = 0.041; primer B, *P = 0.020 by one-sided Student’s t-test. b, dCas9::VP64-mediated elevation of SPR-4 protein levels. Left, representative images of the head region of heterozygous F1 progeny of the strains bearing a spr-4::gfp::spr-4utr transgene. Arrowheads indicate SPR-4::GFP-positive nuclei. Dashed red lines indicate the outline of the worm body. Scale bar, 40 μm. Middle, SPR-4::GFP protein levels are increased by dCAS9::VP64-mediated activation. Values represent mean ± s.e.m. sgRNA(−), n = 5 worms; sgRNA(+), n = 5 worms with 7–38 measurements per worm; *P = 0.022, one-sided Student’s t-test. Right, SPR-4::GFP expression appears in more cells following dCAS9::VP64-mediated activation. Values represent mean ± s.e.m. sgRNA(−), n = 4 worms; sgRNA(+), n = 4 worms. *P = 0.011, one-sided Student’s t-test. Shown is a representative experiment replicated three times. c, Extended lifespan in worms expressing an integrated dCas9::VP64 transgene and sgRNAs targeting the spr-4 promoter (sgRNA(+)) (n = 79 worms) relative to dCas9::VP64-expressing worms in the absence of sgRNAs (sgRNA(−)) (n = 57 worms). P = 5.5 × 10−9, log-rank test. Representative of an experiment replicated six times. d, Lifespans of worms expressing sgRNA targeting the spr-4 promoter in the presence (n = 87 worms) or absence (n = 58 worms) of dCas9::VP64. P = 3.7 × 10−7, log-rank test. Representative of an experiment replicated twice. e, Lifespans of dCas9::VP64-expressing worms in the presence (n = 51 worms) or absence (n = 58 worms) of sgRNAs on the spr-4(tm465) loss-of-function mutant background. P = 0.49, log-rank test. Representative of three independent replicates. f, Overexpression of spr-4 reduces neural excitation. GCaMP imaging was performed in ASH neurons in SPR-4-overexpressing (sgRNA(+)) and control (sgRNA(−)) worms in the lines described in c. Shown are maximum GCaMP fluorescence changes. sgRNA(−), n = 12 worms; sgRNA(+), n = 10 worms. *P = 0.025, Mann–Whitney U-test.
Extended Data Fig. 7 | Lifespan extension by overexpression of spr-4 and inhibition of neural excitation depends on DAF-16. a, Extension of lifespan by overexpression of spr-4 is dependent on DAF-16. Lifespans of worms overexpressing spr-4 (sgRNA(+)::dCas9::VP64) or not overexpressing spr-4 (sgRNA(–); dCas9::VP64) following treatment with daf-16 RNAi or an empty vector control. sgRNA(+) EV (n = 29 worms) versus sgRNA(–) EV (n = 25 worms): P = 2.7 × 10⁻⁴; sgRNA(+) daf-16 (n = 18 worms) versus sgRNA(–) daf-16 (n = 29 worms): P = 0.20 by log-rank test. Representative of four independent replicates. b, Extension of lifespan by the inhibitors of neural excitation ivermectin and nemadipine is DAF-16-dependent. Shown are lifespan determinations for wild-type control and daf-16 (mu86) mutant worms in the presence or absence of nemadipine (2 μM; b) or ivermectin (1 pg ml⁻¹; c). b, WT, n = 69 worms; WT + Nema, n = 51; daf-16, n = 43; daf-16 + Nema, n = 67. WT + Nema versus WT, P = 9.9 × 10⁻⁴; daf-16 + Nema versus daf-16, P = 0.014; log-rank test. c, WT, n = 78 worms; WT + Ivm, n = 77; daf-16, n = 27; daf-16 + Ivm, n = 29. WT + Ivm versus WT, P = 7.3 × 10⁻⁴; daf-16 + Ivm versus daf-16, P = 0.22; log-rank test. Curves are representative of an experiment replicated two (nemadipine) or three (ivermectin) times. d, Inhibition of neural excitation with ivermectin elevates DAF-16 levels. Worms expressing a Daf-16::GFP transgene were treated for 10 days with 1 pg ml⁻¹ ivermectin and assessed by confocal microscopy. Left, total DAF-16::GFP (mean ± s.e.m.). Untreated, n = 19 worms; ivermectin, n = 16 worms. **P = 2.5 × 10⁻³, Mann–Whitney U-test. Right, nuclear DAF-16::GFP. n = 5 worms per group. 50–61 nuclei per worm. *P = 0.013 by Student's t-test. Results are representative of an experiment replicated twice. e, DAF-16 is not required for inhibition of neural excitation by nemadipine. Shown are maximum ASH GCaMP intensity changes for day 2 daf-16(mu86) mutant worms treated for 24 h with 2 μM nemadipine (untreated, n = 16 worms; nemadipine, n = 18 worms), P = 9.4 × 10⁻³, Mann–Whitney U-test. f, DAF-16 is not required for inhibition of neural excitation by ivermectin. Shown are data from day 2 worms treated for 24 h with 1 pg ml⁻¹ ivermectin (control, n = 19 worms; ivermectin, n = 32 worms). P = 0.030, Mann–Whitney U-test.
Extended Data Fig. 8 | See next page for caption.
Extended Data Fig. 8 | SPR-3 and SPR-4 contribute to lifespan extension and gene regulation associated with reduced DAF-2 and insulin–IGF-like signalling. a, Loss of function of SPR-3 and SPR-4 reduces the lifespan extension induced by daf-2 RNAi. Left, representative lifespan analysis of *spr-4(by105);spr-3(ok2525) double mutant and wild-type worms following daf-2 or empty vector control RNAi. WT + EV, *n* = 54 worms; *spr-4* + EV, *n* = 58 worms; WT + *daf-2*, *n* = 26 worms; *spr-4* + *daf-2*, *n* = 54 worms. Right, values represent mean ± s.e.m. per cent lifespan extension (*daf-2* RNAi versus EV control) in the indicated genotypes. WT, *n* = 6 independent experiments; *spr-4*(*by105*), *n* = 3, *P* = 0.017 versus WT; *spr-4(tm465)*, *n* = 4, **P* = 0.0062 versus WT; *spr-3(ok2525)*, *n* = 4, **P* = 0.0018 versus WT; *spr-4(by105);spr-3(ok2525)*, *n* = 4, **P* = 0.0016 versus WT; Students’ *t*-test. See Supplementary Table 22 for individual lifespan data and statistics. b, Lifespan is unaffected by *spr-4* and *spr-3* mutations in a wild-type background. WT, *n* = 50 worms; *spr-3(ok2525)*, *n* = 31; *spr-4(by105);spr-3(ok2525)*, *n* = 32; *spr-4(by105)*, *n* = 34; *spr-4(tm465)*, *n* = 33. There were no reproducibly significant changes by the log-rank test in 3–6 independent experiments per genotype (see Supplementary Table 22). c, Quantification of lifespan extension in *daf-2* single mutant worms shown in Fig. 3b attributable to neuronal expression of *spr-3* and *spr-4*. RNAi was targeted to neurons by neuronal expression of a *sid-1* transgene in otherwise *sid-1-null daf-2(1370) mutants (*daf-2*;*p[neuron];*sid-1*), and compared with untargeted RNAi in *sid-1* wild-type *daf-2(1370)* mutants (*daf-2*). Values represent mean ± s.e.m. lifespan extension relative to the control *sid-1(pk3321);p[neuron];*sid-1* worms treated with empty vector (*n* = 3 independent experiments). Significant lifespan effects were not observed for RNAi in the absence of the *daf-2* mutation. *P* < 0.05; **P** < 0.01 by Student’s *t*-test. d, Gene expression determined by RNA-seq in day 2 adult worms. Differentially expressed genes (rows) and the indicated worm genotypes (columns) were clustered, and gene expression, transformed to a z-score per gene, is represented in a heat map. *n* = 3 independent replicates per genotype. e, Venn diagram illustrating the overlap in differentially expressed genes in *daf-2* single mutant versus WT and *spr-4;*spr-3;*daf-2* triple mutant versus *daf-2* single mutant worms. *P* = 7 × 10<sup>−8</sup>, Fisher’s exact test with a one-sided alternative hypothesis. f, Long-lived *daf-2* mutants do not show an age-related increase in neural excitation. Shown are maximum ASH GCaMP intensity changes in day 1–2 (*n* = 39) and day 14–16 (*n* = 54) *daf-2(e1370)* mutant worms. Note the absence of the age-related increase in excitation observed in wild-type ageing worms (Fig. 1e). *P* = 0.83, Mann–Whitney *U*-test. g, The *spr-4*;*spr-3* double mutation in a wild-type background does not significantly affect neural excitation in ASH neurons. WT, *n* = 15 worms; *spr-4*;*spr-3*, *n* = 15 worms. *P* = 0.62, Mann–Whitney *U*-test. h, DAF-16 does not mediate suppression of neural excitation in the *daf-2* mutant. RNAi against daf-16 was performed in *daf-2(e1370)* mutant worms on a *sid-1(pk3321);p[neuron];*sid-1* background to augment RNAi in neurons (*daf-16* RNAi, *n* = 20 worms, EV control, *n* = 12 worms). *P* = 0.33, Mann–Whitney *U*-test. i, Descriptions of the genes targeted by RNAi in Fig. 4d.
Extended Data Fig. 9 | See next page for caption.
Extended Data Fig. 9  |  Regulation of DAF-16 by SPR-3 and SPR-4.

**a.** Reduced DAF-16 activation in spr-4;spr-3 mutants following daf-2 RNAi. Left confocal image, day 10 worms of the indicated genotypes expressing an integrated Daf-16::GFP transgene and treated with daf-2 RNAi or empty vector control since day 1 of adulthood. Images are maximum intensity z-projections. Scale bar, 40 μm. Left bar graph, mean ± s.e.m. GFP intensity in the peri-pharyngeal regions of spr-4;spr-3 double mutants relative to wild-type controls for a representative experiment replicated four times (see Methods for details of analysis). n = 8–12 worms per replicate. **P = 5.2 × 10^{-5},** Welch’s t-test. Right confocal image, higher-magnification views of DAF-16::GFP and DAPI-labelled nuclei. Images are magnified confocal z-planes. Scale bar, 10 μm. Right bar graph, mean ± s.e.m. nuclear GFP intensity relative to the WT-EV control. n = 5 worms per genotype and 51–89 nuclei per worm. *P = 0.016, **P = 5.5 × 10^{-4} by ANOVA with post hoc Tukey test. Values and images are representative of an experiment replicated three times.

**b.** Gene expression determined by RNA-seq in adult day 10 worms. Differentially expressed genes (rows) and replicates of the indicated worm genotypes (columns) were clustered, and gene expression, transformed into a z-score per gene, is represented in a heat map. n = 3 independent replicates per genotype. c. Venn diagram illustrating the overlap of differentially expressed genes in day 10 daf-2 versus wild-type and spr-4;spr-3;daf-2 versus daf-2 worms. P = 4 × 10^{-12}, Fisher’s exact test with a one-sided alternative hypothesis.

**d.** Overlap of class 1 daf-16 target genes (see Methods) with genes downregulated in day 10 spr-4;spr-3;daf-2 triple mutants relative to daf-2 single mutants. P values calculated using a hypergeometric distribution (see Methods). n.s., P = 0.99.

**e.** Ivermectin increases DAF-16::GFP levels in spr-4;spr-3 worms following daf-2 RNAi. Left, confocal imaging of GFP fluorescence in ivermectin-treated (10 pg ml^{-1}) and untreated worms. The red dashed lines indicate the worm body. Right, quantification of DAF-16::GFP (mean GFP intensity ± s.e.m., WT/Untreated, n = 12; WT/Ivermectin, n = 10; spr-4;spr-3/Untreated, n = 10; spr-4;spr-3/Ivermectin, n = 10. **P = 4.6 × 10^{-4} (spr-4;spr-3 versus WT/untreated), P = 2.6 × 10^{-4} (spr-4;spr-3/ivermectin versus spr-4;spr-3/untreated) by Mann–Whitney U-test with multiple testing correction by Holm’s method. Shown is a representative experiment replicated three times.
Extended Data Fig. 10 | Coregulation of FOXO1 and REST in the ageing brain and modulation by glutamatergic signalling. a, Linear regression analysis of REST and FOXO mRNA levels in the prefrontal cortex of 174 cognitively intact individuals (age ≥60 years) from the CMC cohort determined by RNA-seq. P values derived from a t-test for the slope of the regression line and Bonferroni-corrected across all expressed genes. b, Colocalization of REST and FOXO1 in neurons of the aged human prefrontal cortex. Confocal immunofluorescence microscopy was performed in human prefrontal cortex using antibodies against REST (green, rabbit polyclonal; Bethyl), FOXO1 (red, goat polyclonal; LS-Bio) and the neuronal marker MAP2 (grey, chicken polyclonal; Abcam). Scale bar, 40 μm. The image shown is representative of immunofluorescence labelling performed in 30 individuals. See Supplementary Table 20 for additional information on antibodies. c, Inhibition of glutamatergic signalling in mouse cortical neuronal cultures elevates FOXO1 levels. Left, primary mouse cortical neuronal cultures treated with kynurenic acid (KYN, 5 μM), AP5 (50 μM), NBQX (2 μM) or vehicle control were analysed by confocal immunofluorescence for FOXO1 or MAP2 and labelled with DAPI. Boxed areas are magnified in the lower three rows. Note that most FOXO1 in cultured neurons is cytoplasmic, but a detectable nuclear component overlaps with DAPI. Scale bar, 40 μm. Right, quantification (mean ± s.e.m.) of total and nuclear FOXO1 levels in MAP2-positive neurons. Control, n = 200; KYN, n = 326; AP5, n = 148; NBQX, n = 197. FOXO1 total/KYN, **P = 2.1 × 10−8; FOXO1 nuclear/KYN, **P = 1.1 × 10−8; FOXO1 total/NBQX, **P = 8.8 × 10−13; FOXO1 nuclear/NBQX, **P = 5.2 × 10−6; Mann–Whitney U-test with multiple testing correction by Holm’s method. Shown is a representative experiment replicated three times.