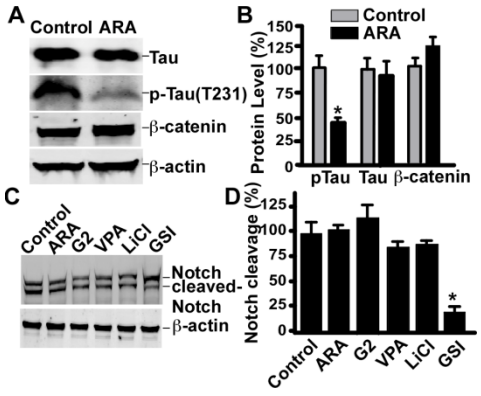
Online Supplementary Data

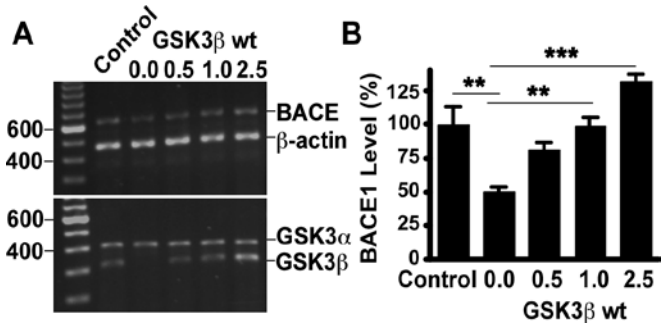
Supplemental Table 1. The Effect of AR-A014418 on Protein Kinases

Kinase	Kinase full name	Kinase activity (%) Control	5 μM ARA
GSK3α	Glycogen synthase kinase 3α	100.0±2.2	2.0±0.1
GSK3β	Glycogen synthase kinase 3β	100.0±4.2	4.2±0.2
AMPKα	5'-AMP-activated protein kinase α1	100.0±1.4	99.7±3.8
AMPKα2	5'-AMP-activated protein kinase α2	100.0±0.3	102.8±1.0
CDK2/cyclin A1	cyclin dependent protein kinase 2	100.0±1.3	94.8±0.9
CDK3/cyclin E1	cyclin dependent protein kinase 3	100.0±1.3	63.9±1.8
CDK4/cyclin E1	cyclin dependent protein kinase 4	100.0±5.3	92.9±2.1
CDK5/p25	cyclin dependent protein kinase 5	100.0±0.8	96.0±1.4
DYRK1A	Dual-specificity tyrosine- (Y)-phosphorylation regulated kinase 1A	100.0±1.4	95.8±1.4
HIPK1	Homeodomain- interacting protein kinase 1	100.0±2.3	99.2±0.8
HIPK3	Homeodomain- interacting protein kinase 3	100.0±1.5	94.8±3.9
PAK6	Ser/thr-protein kinase PAK 6	100.0±22	103.0±1.4
PKAα	cAMP-dependent protein kinase, alpha-catalytic subunit	100.0±0.3	102.7±0.8
PKCζ	Protein kinase C, zeta type	100.0±0.6	101.0±0.9

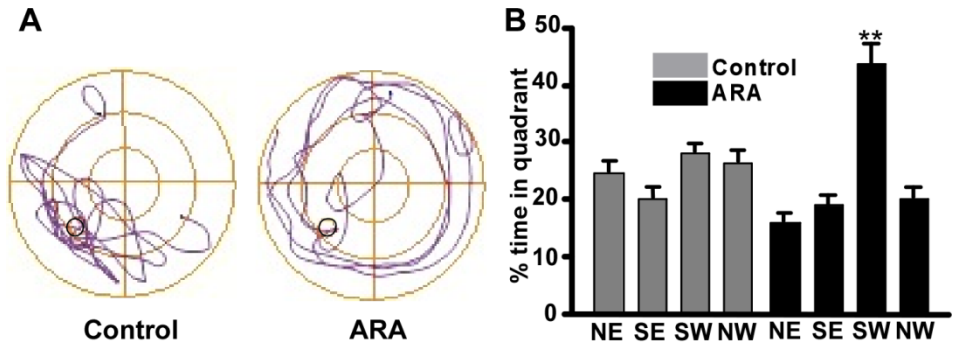
We profiled the AR-A014418 against GSK3α/β and against protein kinases that are structurally – related as determined by algorithms at Kinexus Bioinformatics. The kinase assay was performed at Kinexus Bioinformatics following well-established protocol. The assay conditions for the various protein kinase targets were optimized to give high signal-to-noise ratio. The detailed protocol could be obtained via www.kinexus.ca. Briefly, a radioisotope assay format was used for profiling evaluation of the kinase targets. Protein kinase assays were performed at 30°C for 30 minutes in the presence of 100 mM ^{33}P-ATP with 5 μM AR-A014418 or 10% DMSO. The final volume is 25 ml. After 30 minutes, the assay was halted by spotting 10 ml of the reaction mixture onto Multiscreen phosphocellulose P81 plate. The P81 plates was washed 3 times with phosphoric acid solution followed by counting using a Trilux scintillation counter. The profiling of AR-A014418 demonstrated potent GSK3 inhibition and did not significantly the activity levels of other kinases.



Supplemental Figure 1. Effect of AR-A014418 on Tau phosphorylation and Notch cleavage. **(A and B)** AR-A014418 treatment in APP23/PS45 mice reduced GSK3-dependent tau phosphorylation, without affect total tau levels. The protein level of beta-catenin were not affected with GSK3 inhibition. The bars represent mean±S.E.M. n=4 per group. *$p<0.05$ by Student's t-test. **(C)** HEK293 cells stably expressing Notch construct was treated with GSK3 inhibitors ARA, GS2, and LiCl, as well as valproic acid and γ-secretase inhibitor L658,458. **(D)** GSK3 inhibition with ARA, G2, and LiCl did not affect Notch cleavage. Valproic acid, which could inhibit GSK3 activity also did not affect Notch cleavage. Significant reduction of Notch cleavage by γ-secretase inhibitor. The bars represent mean±S.E.M. n=4 per group. *$p<0.05$ by Student's t-test.



Supplemental Figure 2. GSK3β increased BACE1 mRNA expression. (A) SH-SY5Y cells were co-transfected with GSK3β siRNA and increasing amounts of wt-GSK3β expression plasmids or GFP plasmids for 72 hrs. Cells were harvested with Tri-A reagent and RNA extracted as previously described. Thermoscript Reverse Transcription kit (Invitrogen) was used to synthesize the first strand cDNA from an equal amount of RNA following the manufacturer's instruction. The newly synthesized cDNA templates were further amplified via Platinum *Taq* DNA polymerase in a 20 μL reaction. BACE1, GSK3α, GSK3β, APP, and PS1 genes were amplified with gene-specific primers (details in Materials and Methods section). β-actin was used as control. The samples were resolved and analyzed on a 1.2% agarose gel. (B) Quantification of BACE1 mRNA levels. GSK3β knockdown reduced BACE expression and this effect was rescued by addition of increasing amount of exogenous GSK3β cDNA. With GSK3β knockdown, BACE1 expression is 50.2±3.3% of scramble siRNA. Simultaneously transfecting 0.5, 1, 2.5 μg of GSK3β plasmid increased BACE1 expression to 81.3±5.9%, 98.8±6.7%, and 132.4±5.4%, respectively. The bars represent mean±S.E.M. n=4. **$p<0.01$, ***$p<0.001$ by ANOVA followed by Tukey's post hoc analysis.



Supplemental Figure 3

Supplemental Figure 3. AR-A014418 treatment rescued memory deficits in APP23/PS45 mice. APP23/PS45 double transgenic mice at the age of 6 weeks were treated with 5 mg/Kg AR-A014418 via intraperitoneal injection daily for 1 month. The mice were then subjected to the water maze test to assess for changes in learning and memory functions. The mice did not display any sensorimotor deficits with AR-A014418 treatment as indicated the visible platform test. AR-A014418 improved learning and memory abilities as seen in the hidden platform test and probe trial. The probe trial occur during the last day of testing where the platform was removed and the mice were allowed 60 seconds to recall where the platform was originally placed. **(A)** Thigmotaxic swim pattern of a control-treated and ARA-treated APP23/PS45 mice during the probe trial. The sham-treated animals cannot remember where the platform was placed, where as ARA-treatment mice swim closely to the area where the platform used to be (indicated by the black circle). **(B)** ARA-treated mice also remembered the original location of the platform as indicated by spending significantly more time in the in southwest quadrant. The bars indicate mean±S.E.M. (n=26 mice total, 14 ARA treated and 12 sham treated). **$p<0.01$ by ANOVA followed by Tukey's post hoc analysis.