# Supporting Information

## Keskin et al. 10.1073/pnas.1708106114

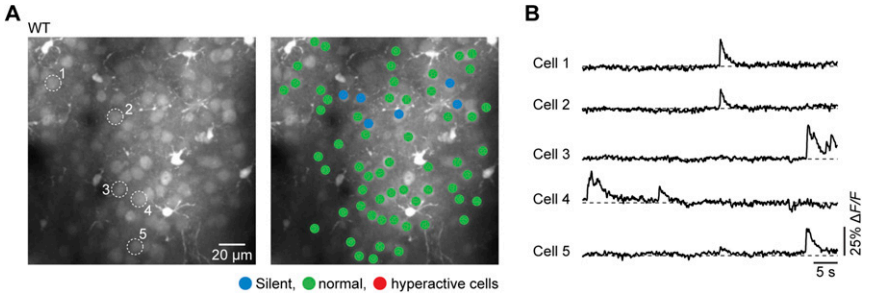


**Fig. S1.** Normal activity of cortical neurons in WT mice in vivo. (*A*, *Left*) Representative in vivo two-photon fluorescence image of layer 2/3 cell population in the frontal cortex and corresponding activity map obtained from a WT mouse (*A*, *Right*). In the activity map, neurons are color-coded according to their activity status. (*B*) Activity traces from five example neurons marked in *A*, *Left*.

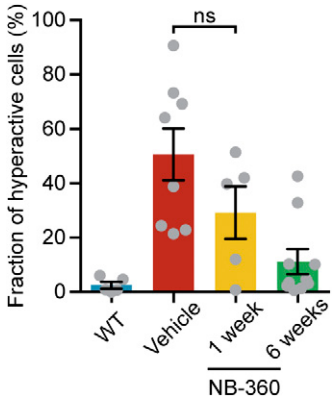


**Fig. S2.** Fractions of hyperactive neurons after short-term treatment with BACE inhibitor. Fractions of hyperactive neurons relative to all imaged neurons in WT mice (blue bar; $n = 5$), untreated APP23xPS45 mice (red bar; $n = 8$), and APP23xPS45 mice after short-term (yellow bar; $n = 5$) and long-term (green bar; $n = 10$) NB-360 treatment (2.48 ± 1.20% for WT vs. 50.60 ± 9.53% for control vs. 29.22 ± 9.69% for short-term treatment vs. 11.15 ± 4.62% for long-term treatment; $P > 0.05$, control vs. short-term treatment). Gray circles represent individual animals. ns, not significant. Error bars represent mean ± SEM.

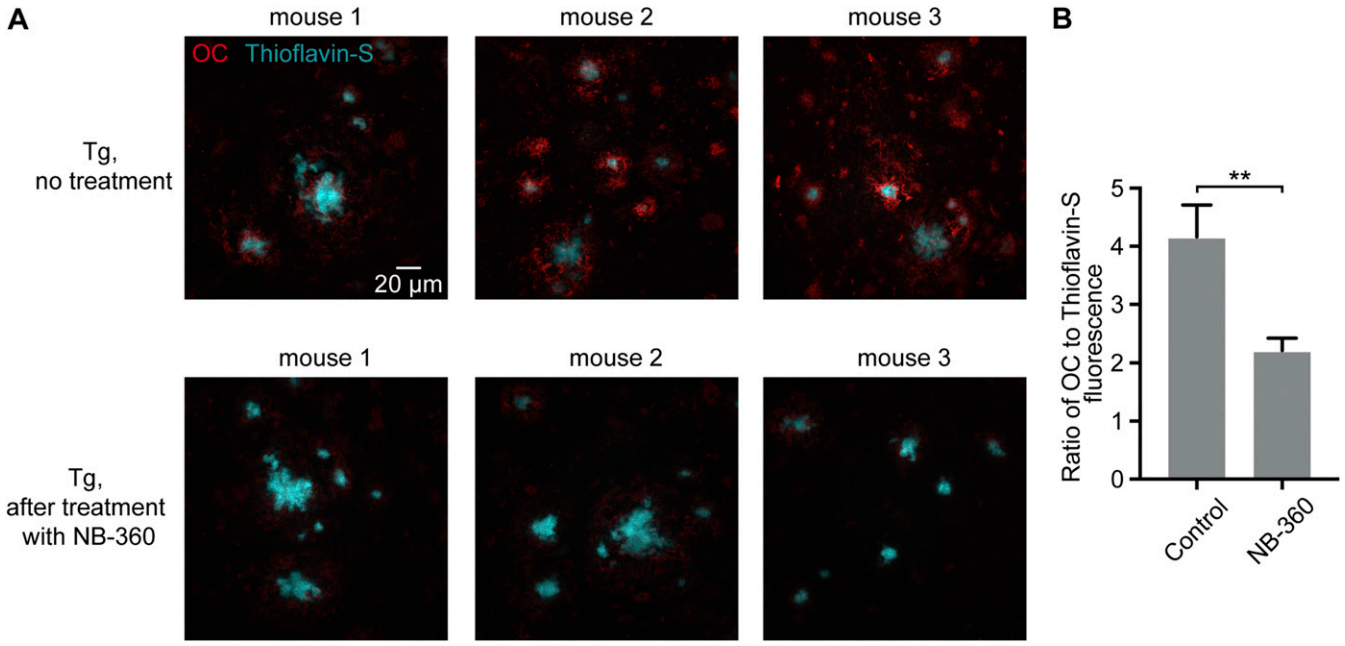


**Fig. S3.** BACE inhibitor treatment reduces the amount of prefibrillar Aβ in the periphery of plaques. (*A*) Confocal images of cortical sections from untreated (*Top*) and NB-360-treated (*Bottom*) APP23xPS45 mice double-labeled with OC antibody (red) and thioflavin-S (cyan). (*B*) Quantification of the ratio of $OC^+$ prefibrillar Aβ to thioflavin-$S^+$ plaque cores in the untreated mice (control; $n = 4$) and treated mice (NB-360; $n = 6$). There was a highly significant reduction in the amount of prefibrillar Aβ around the plaques after treatment with NB-360 (4.16 ± 0.55 for control vs. 2.22 ± 0.21 for NB-360; two-sample $t$ test, $t = 3.8$; df = 8; $P = 0.0050$). $^{**}P < 0.01$. Error bars represent mean ± SEM.

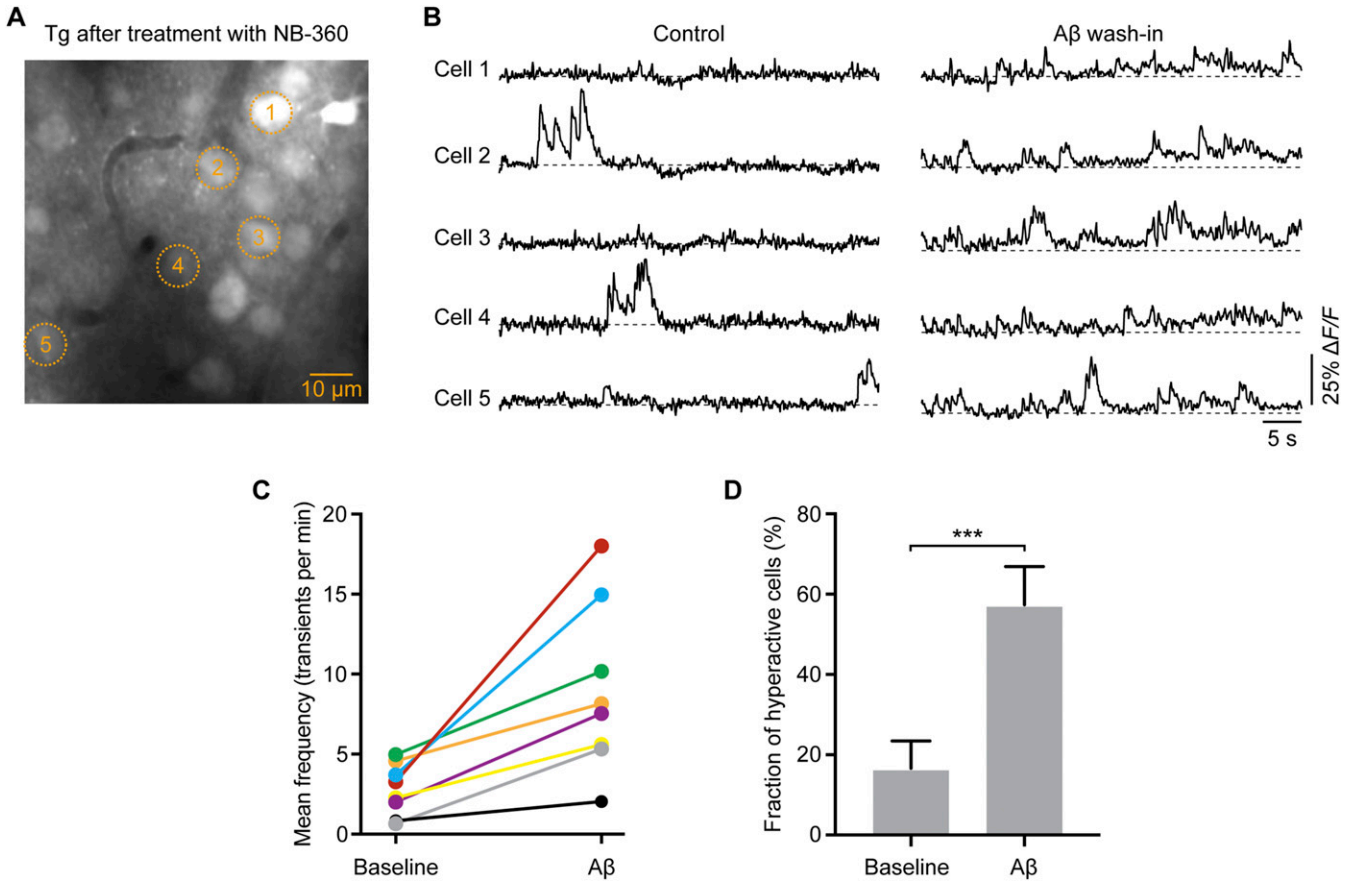


**Fig. S4.** Recurrence of neuronal hyperactivity after topical application of Aβ dimers to the cortex of NB-360–treated APP23xPS45 mice. (*A*) In vivo two-photon fluorescence image of layer 2/3 neurons in a NB-360–treated APP23xPS45 mouse. (*B*) $Ca^{2+}$ transients of five neurons, marked in *A*, before (baseline) and 60 min after superfusion of the cortex with AβS26C cross-linked dimers (1 μM). (*C*) Average rate of $Ca^{2+}$ transients in all recorded cortical regions ($n = 8$ regions in three mice) before and after Aβ application (2.79 ± 0.57 transients/min for baseline vs. 8.98 ± 1.86 transients/min for Aβ; paired *t* test, $t = 3.883$; df = 7; $P = 0.0060$). (*D*) Fractions of hyperactive neurons from all recorded cortical regions ($n = 8$ regions in 3 mice) before and after Aβ application (16.62 ± 6.85% for baseline vs. 57.35 ± 9.58% for Aβ; paired *t* test, $t = 5.628$; df = 7; $P = 0.0008$). ***$P < 0.001$. Error bars represent mean ± SEM.

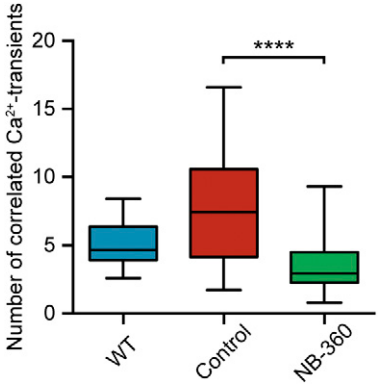


**Fig. S5.** BACE inhibition reduces hypersynchrony of neurons. There was a significantly higher number of correlated $Ca^{2+}$ transients in untreated APP23xPS45 mice (red; $n = 64$ recording sites in eight mice) relative to WT mice (blue; $n = 47$ recording sites in five mice), and a strong decrease in abnormal correlation in NB-360–treated APP23xPS45 mice (green; $n = 81$ recording sites in 10 mice) [$F(2,198) = 39.79$, $P < 0.0001$; Tukey's post hoc comparisons: $P < 0.0001$ for WT vs. control and $P < 0.0001$ for control vs. NB-360). In the boxplot, the top bar is the maximum observation, the lower bar is the minimum observation. The box extends from the 25th percentile to the 75th percentile, with the middle bar as the median. ****$P < 0.0001$.

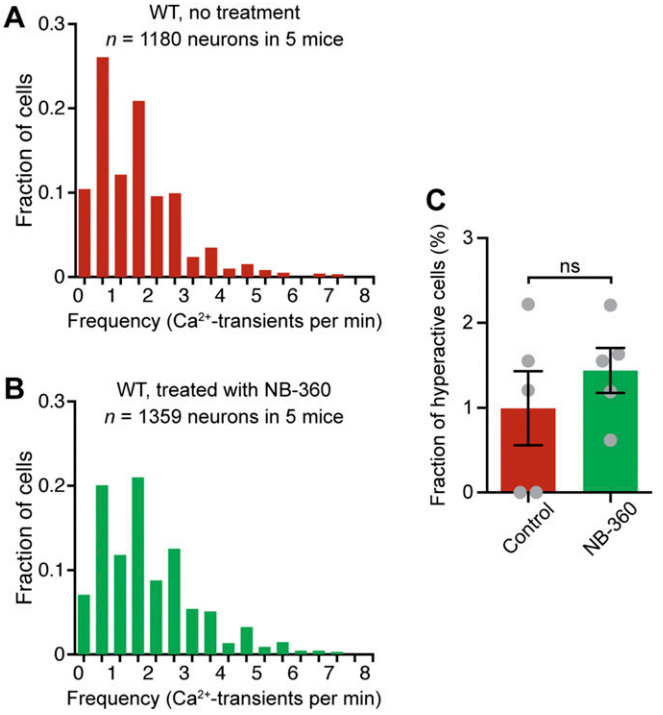


**Fig. S6.** No significant change of neuronal activity patterns after BACE inhibitor treatment in WT mice. (*A* and *B*) Summary histograms of activity in all recorded neurons from untreated (*A*) and NB-360–treated (*B*) WT mice. (*C*) Fractions of hyperactive neurons in untreated (red bar, $n = 5$) and treated (green bar, $n = 5$) WT mice (1.00 ± 0.44% for untreated vs. 1.44 ± 0.26% for treated; two-sample *t* test, $t = 0.87$; df = 6.54; $P = 0.4152$). Gray circles represent individual animals. ns, not significant. Error bars represent mean ± SEM.

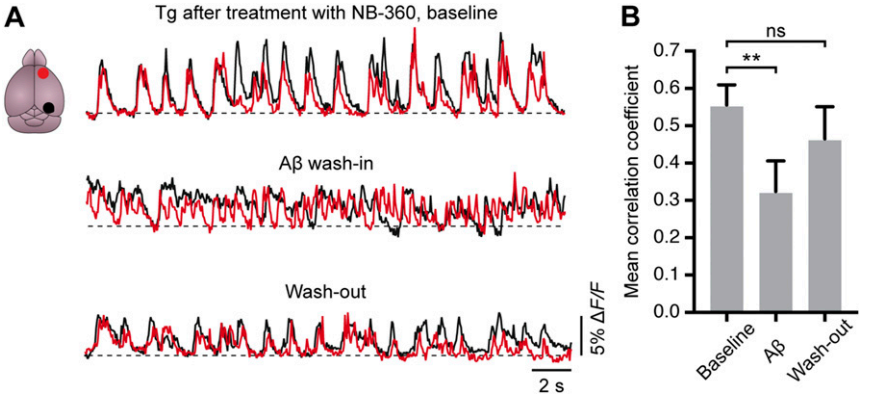


**Fig. S7.** Reintroducing Aβ into the cortex reversed the beneficial effects of BACE inhibition on long-range functional connectivity. (*A*) Representative traces from frontal (red) and occipital (black) cortex of an NB-360–treated APP23xPS45 mouse before (*Top*), during (*Middle*), and after (*Bottom*) superfusion of the cortex with synthetic Aβ 1–40/1–42 (1 μM). (*B*) Summary graph of the average correlation strength between the frontal and occipital cortex in NB-360–treated APP23xPS45 mice ($n = 5$ mice) under the three conditions shown in *A* (0.56 ± 0.05 for baseline vs. 0.32 ± 0.08 for Aβ vs. 0.47 ± 0.09 for washout). There was a significant effect of the treatment [linear mixed-effects model, $F(2,11) = 7.07$, $P = 0.011$], and the difference between the baseline condition and the Aβ wash-in was highly significant [$F(1,11) = 14.0$, $P = 0.0032$]. $^{**}P < 0.01$. ns, not significant. Error bars represent mean ± SEM.

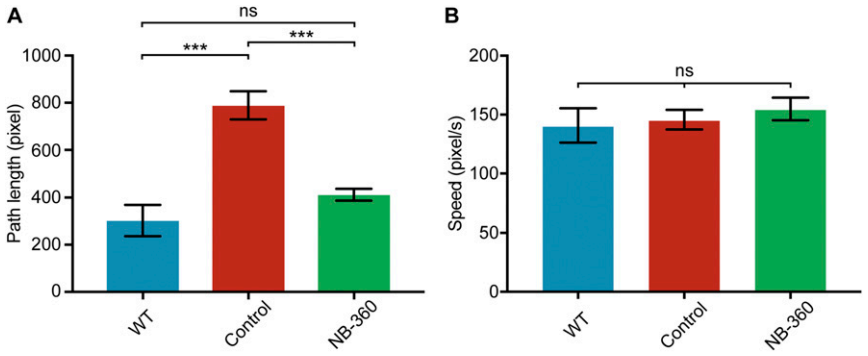


**Fig. S8.** Different escape latencies in WT as well as untreated and treated APP23xPS45 mice are due to the swimming trajectories, not to differences in swimming speed. (*A* and *B*) Bar graphs showing the path length (*A*) and the swimming speed (*B*) of WT mice (blue, $n = 6$), untreated APP23xPS45 mice (red, $n = 5$), and APP23xPS45 mice after 6 wk of treatment with NB-360 (green, $n = 5$). Path length: 302.3 ± 66.4 pixels for WT vs. 789.2 ± 59.9 pixels for control vs. 412.1 ± 25.4 pixels for NB-360–treated [$F(2,13) = 20.68$, $P < 0.0001$; Tukey's post hoc comparisons: $P < 0.0001$ for WT vs. control, $P = 0.0013$ for control vs. NB-360, $P = 0.3668$ for WT vs. NB-360]. Swimming speed: 140.8 ± 14.6 pixels for WT vs. 145.7 ± 8.4 pixels for control vs. 154.7 ± 9.6 pixels for NB-360–treated [$F(2,13) = 0.3577$, $P = 0.7059$; Tukey's post hoc comparisons: $P = 0.9522$ for WT vs. control, $P = 0.8635$ for control vs. NB-360, $P = 0.6854$ for WT vs. NB-360. ***$P < 0.01$]. ns, not significant. Error bars represent mean ± SEM.

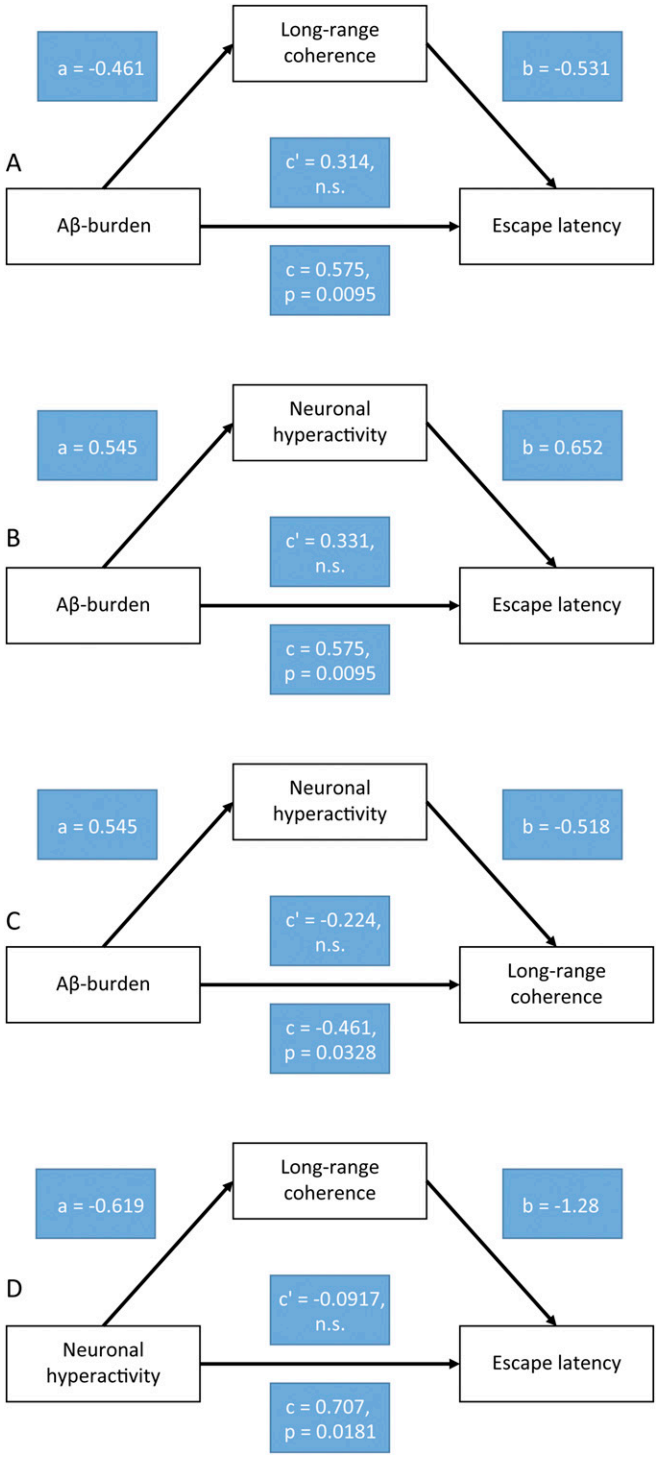


**Fig. S9.** Mediation analysis for the model presented in Fig. 4*E*. Mediation compares the overall effect of a causal variable on an outcome variable with the direct effect computed in the presence of a mediating variable. Mediation occurs when the indirect path through the mediating variable has a significant effect on the outcome variable, so that the direct effect is smaller (or more generally different) than the overall effect. Complete mediation occurs when the direct effect is null. (*A*–*D*) Mediation analyses between two of the quantities presented in Fig. 4*E*, with an intermediate mediating variable. All four possible combinations of causal variable, outcome variable, and mediating variable were tested. The regressions were performed on normalized variables (mean 0, SD 1). The overall effect is given by the coefficient c, and the direct effect is given by coefficient c′. The indirect path through the mediating variable is given by coefficients a and b. In all cases, the indirect path had a significant effect (permutation test, $P < 0.05$ for all cases), but the direct effect (measured by c′) was not significant, suggesting complete mediation. Thus, these results are consistent with the scheme shown in Fig. 4*E*. (*A*) Aβ plaque burden and escape latency, mediated by long-range coherence. (*B*) Aβ plaque burden and escape latency, mediated by neuronal hyperactivity. (*C*) Aβ plaque burden and long-range coherence, mediated by neuronal hyperactivity. (*D*) Neuronal hyperactivity and escape latency, mediated by long-range coherence. Aβ burden, Aβ plaque burden; hyperactivity, fraction of hyperactive cells; long-range coherence, coherence of slow-wave activity between the frontal cortex and occipital cortex; escape latency, mean latency to the platform on day 5 of the water maze task.