Common Postsynaptic Mechanisms for Developmental Cognitive Deficits and AD. Despite the strong correlation between AD cognitive deficits and synaptic marker loss, arguing for synaptic defects underlying cognitive decline, the molecular basis for these cognitive deficits has been unclear. Many previous reports on AD and animal models focused on presynaptic synaptophysin deficits. Synaptophysin knockout mice exhibited no deficits in synaptic plasticity, long-term potentiation or cognitive function, suggesting there can be compensation for synaptophysin loss; although synaptophysin is a useful marker for underlying synaptic defects, synaptophysin loss per se did not seem to account for cognitive and synaptic plasticity deficits in AD. Therefore, we sought to gain insight into potential synaptic defect mechanisms by investigating the overlap between AD cognitive deficit syndromes with known genetic causes (Supplementary Fig. 1). Human developmental cognitive deficits (mental retardation syndromes including Down’s syndrome) overlap AD and AD animals in the common compartmental arena of dendritic spine defects. That increased APP dosage can lead to both AD and Down’s syndrome cognitive deficits makes this comparison even more intriguing. DS appears to have core similarities to mental retardation, AD and AD transgenic mice with respect to early loss and degeneration of dendrites and spines, and accompanying cognitive deficits (Supplementary Fig. 1), strengthening the hypothesis of a mechanistic connection between developmental cognitive disorders and AD. Because PAK mutation causes mental retardation, and large PAK deficits occur in AD, PAK seems to be the missing link to lead to spine defects in these cognitive disorders.

PAK Inhibition with PAK18 Peptide Causes Drebrin Loss and Cofilin Pathology in Hippocampal Neurons. The parallel changes in PAK, cofolin and drebrin in AD, APPswe transgenic mice and Aβ1-42 oligomer-treated hippocampal neurons raise the question whether the large PAK deficits are causally related to the cofolin pathology and drebrin loss. To address this question in vitro, we treated cultured hippocampal neurons with a PAK inhibitor, PAK18, or an inactive mutant peptide, PAK18 R192A. Incubation of primary neurons with PAK18, but not R192A, resulted in a significant reduction in pPAK levels (31% ± 3 compared to control, * P < 0.05) and a loss in drebrin levels (64% ± 11 compared to control, * P < 0.05) in hippocampal neurons (Supplementary Fig. 2). PAK18 peptide treatment also induced a marked loss in the levels of drebrin bound to actin as shown by immunoprecipitation with β-actin. The loss of drebrin was accompanied by persistent punctate cofolin labeling, resembling the cofolin rods induced by neurodegenerative stimuli such as ATP depletion, peroxide and glutamate (Supplementary Fig. 2) 37. Collectively, these results argue that the peptides PAK18 and the negative control R192A are
valid tools for inhibiting PAK, and that inhibition of PAK activity is sufficient to drive both drebrin loss and coflin pathology in vitro.

**PAK Signaling Pathway in Regulation of Actin Dynamics.** Supplementary Fig. 3 summarizes the PAK signaling pathway and indicates the possible sites of Aβ and PI3 kinase intervention. PAK is densely concentrated at peri-synaptic locations where it phosphorylates and activates LIM kinase, which in turn phosphorylates ser3 of the actin-binding protein coflin and prevents its actin-binding and actin-severing activity (Supplementary Fig. 3). When PAK activity is reduced, coflin is active and bound to actin, which, according to our in vitro coflin assay, removes drebrin off actin. This could be due to direct competition because homologous actin-binding domains are shared by coflin and drebrin. A second or alternative possible mechanism for coflin-induced drebrin-actin dissociation involves conformational changes in coflin-bound actin. Drebrin loss in both AD and Down’s syndrome and in normal aging would therefore be predicted as a consequence of reduced PAK activity and coflin pathology. Coflin bound to actin is found in pathological intracellular inclusions of Hirano bodies which are prominent in AD. Although it is unclear whether Hirano bodies are directly involved in AD progression, these intracellular inclusions might cause cytoskeletal and morphological changes leading to altered neuritic transport and synaptic dysfunction. Recent evidence suggests coflin exists in two forms, monomers and dimers or oligomers. The requirement of oxidizing conditions for the formation of coflin oligomers and actin bundles provides a link between oxidative stress and coflin pathology, both of which are prominent characteristics in AD. Our observation that coflin pathology is co-localized with caspase-activity is consistent with our previous reports of similar co-localization of caspase activation and Hirano pathology in AD and focal dendritic caspase activation associated with drebrin loss. This co-localization can now be accounted for because dephospho-cofilin migrates to mitochondria and induces cytochrome C release and caspase activation.

