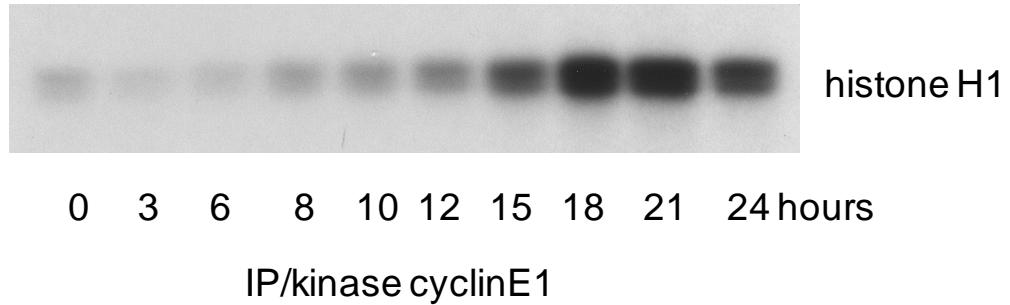
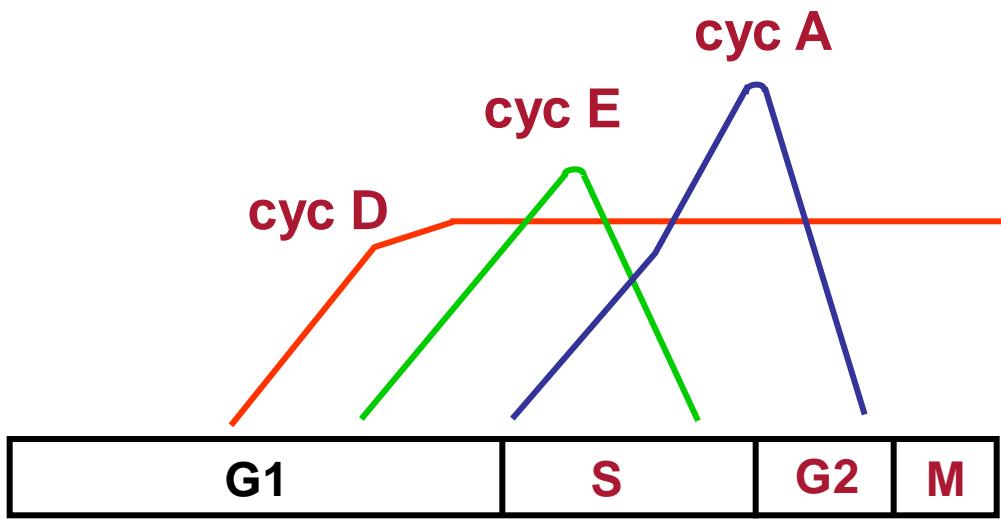
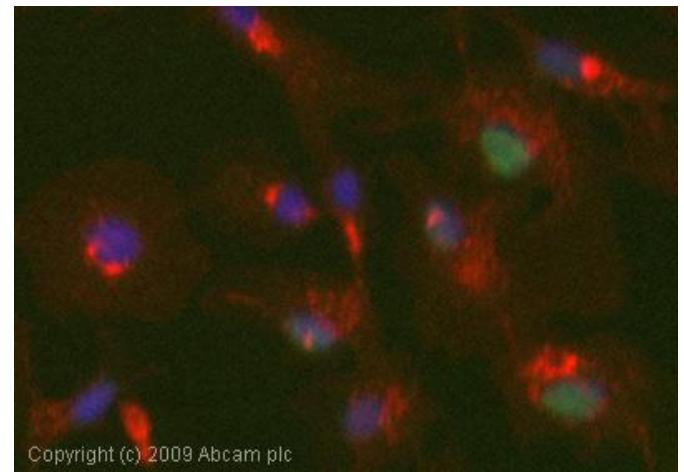


Cyclin E



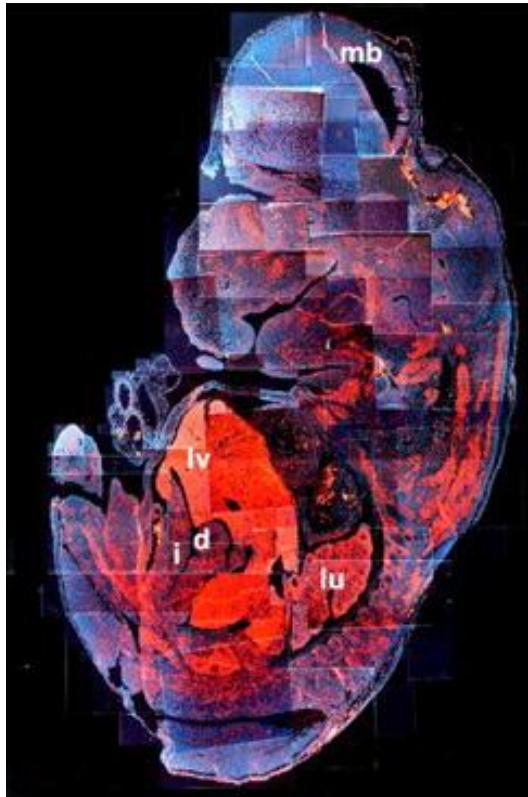
Immunofluorescence staining of cyclin E1



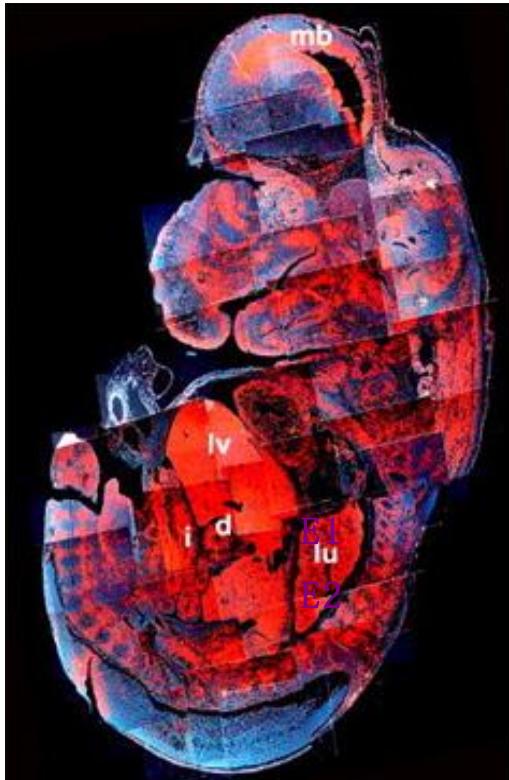
Green: cyclin E1 staining
Blue: DAPI

Expression of E-cyclins

Cyclin E1

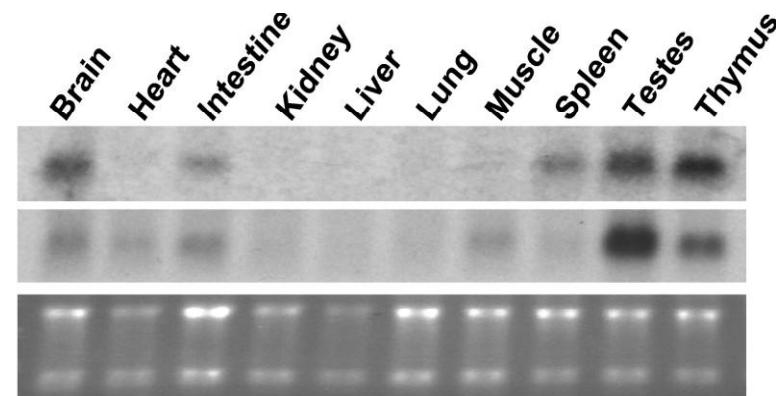


Cyclin E2



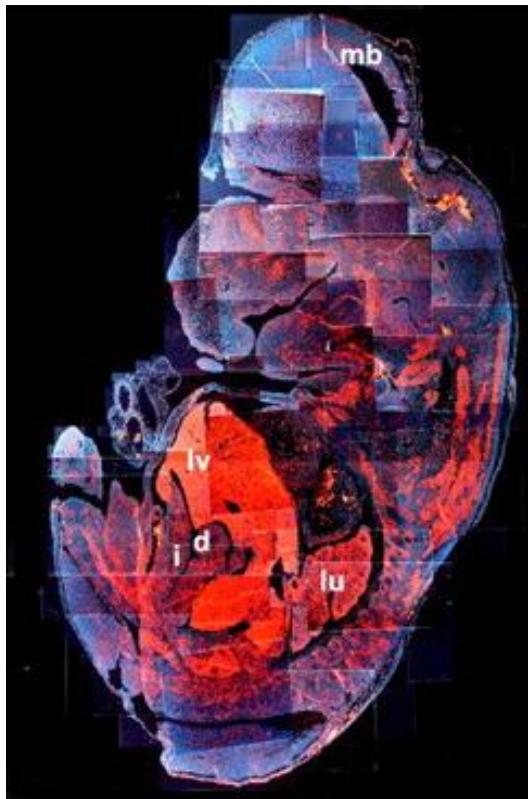
@ E13.5

(Geng et al., PNAS 2001)



Expression of E-cyclins

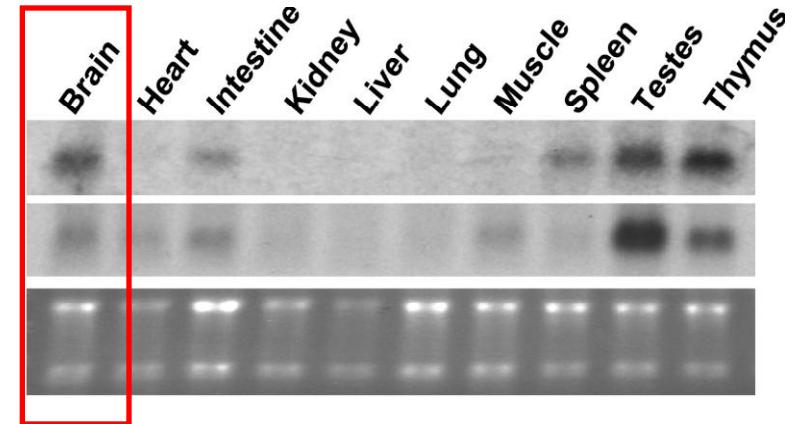
Cyclin E1



Cyclin E2

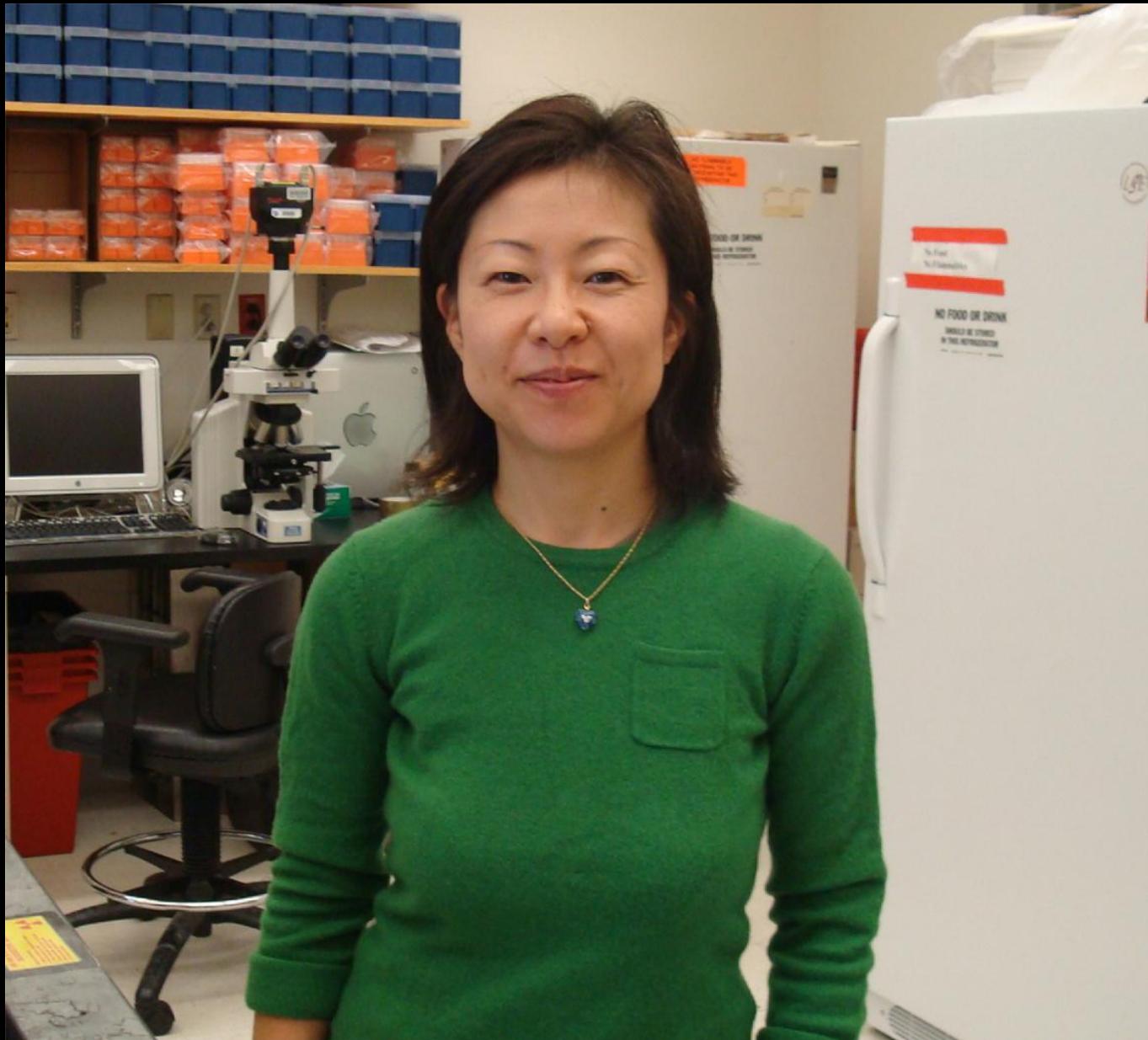


@ E13.5

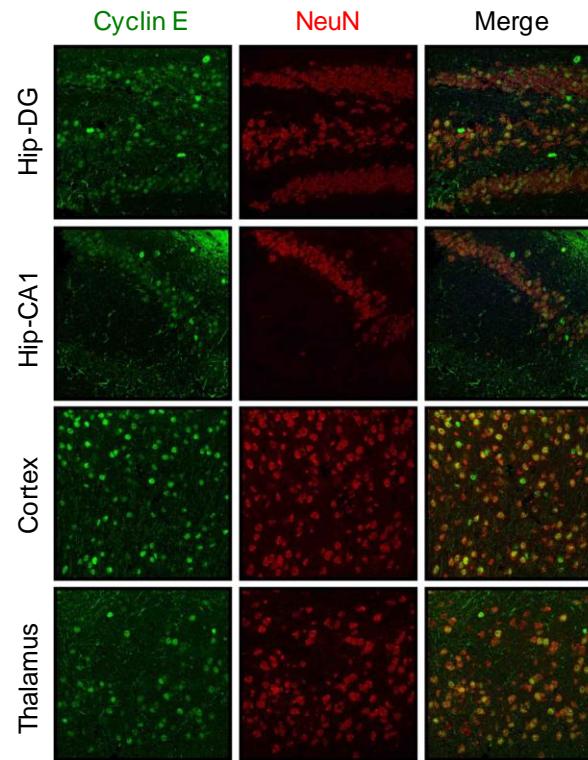
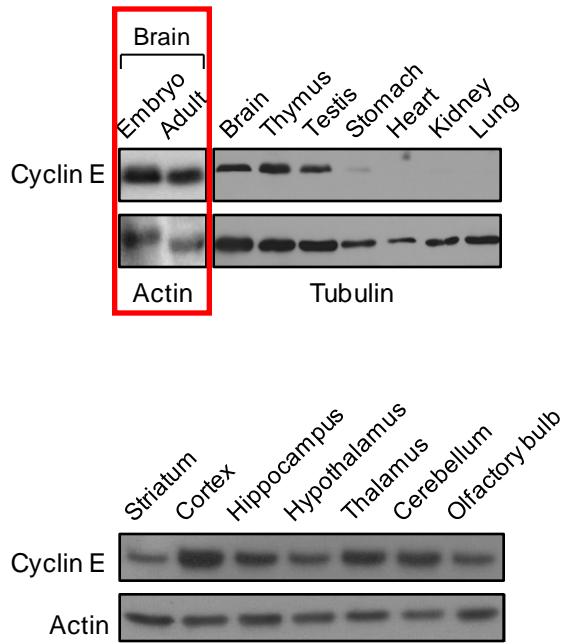


(Geng et al., PNAS 2001)

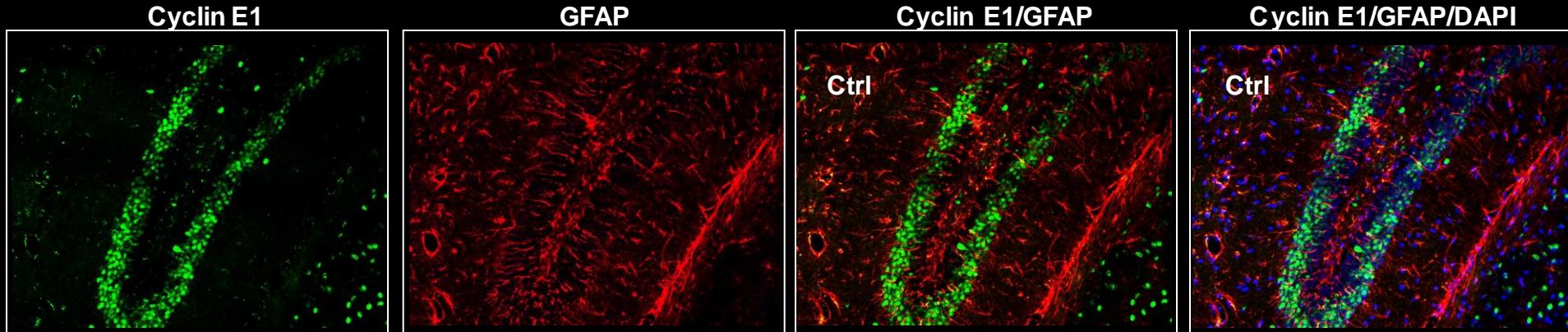
Junko Odajima



Expression of E-cyclins in postmitotic neurons

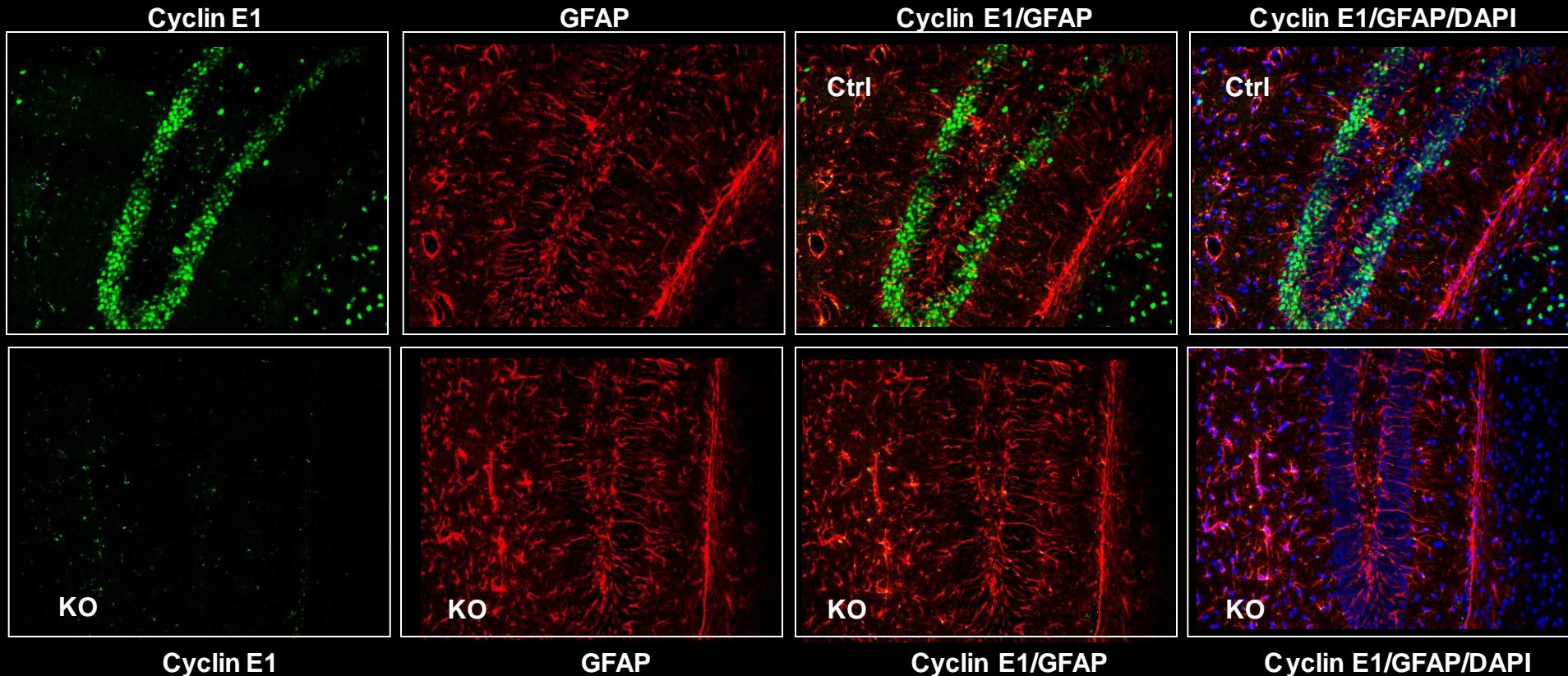


Expression of E-cyclins in postmitotic neurons



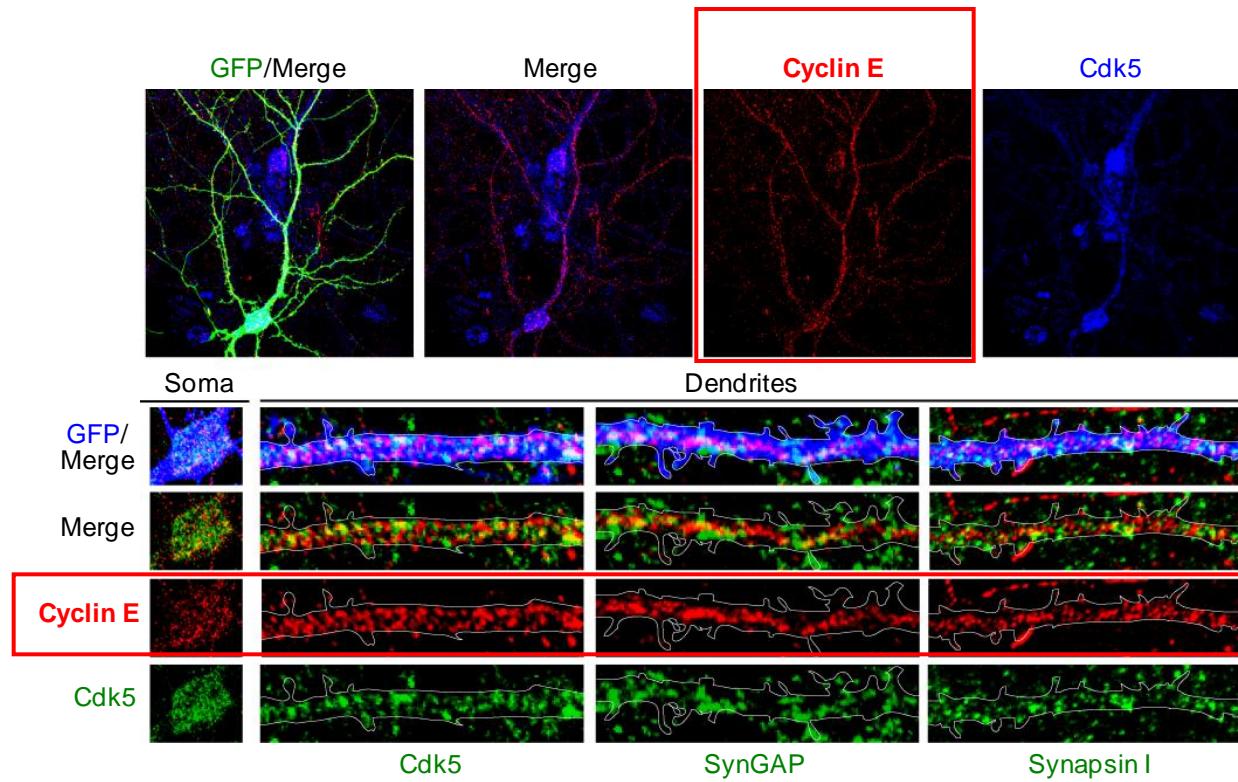
Adult (P33) Hippocampus – Dentate gyrus (20x)

Expression of E-cyclins in postmitotic neurons



Adult (P33) Hippocampus – Dentate gyrus (20x)

Expression of E-cyclins in postmitotic neurons



Cells expressing tagged protein



Wildtype control cells

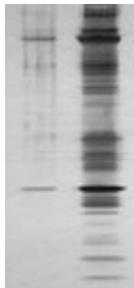
lysate

lysate

Flag

HA

WT tag



Silver stained
quality control gel

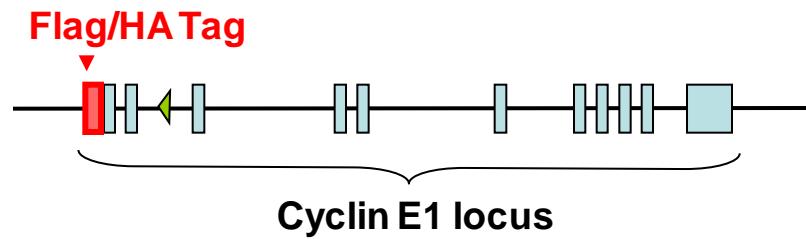
HA

Flag

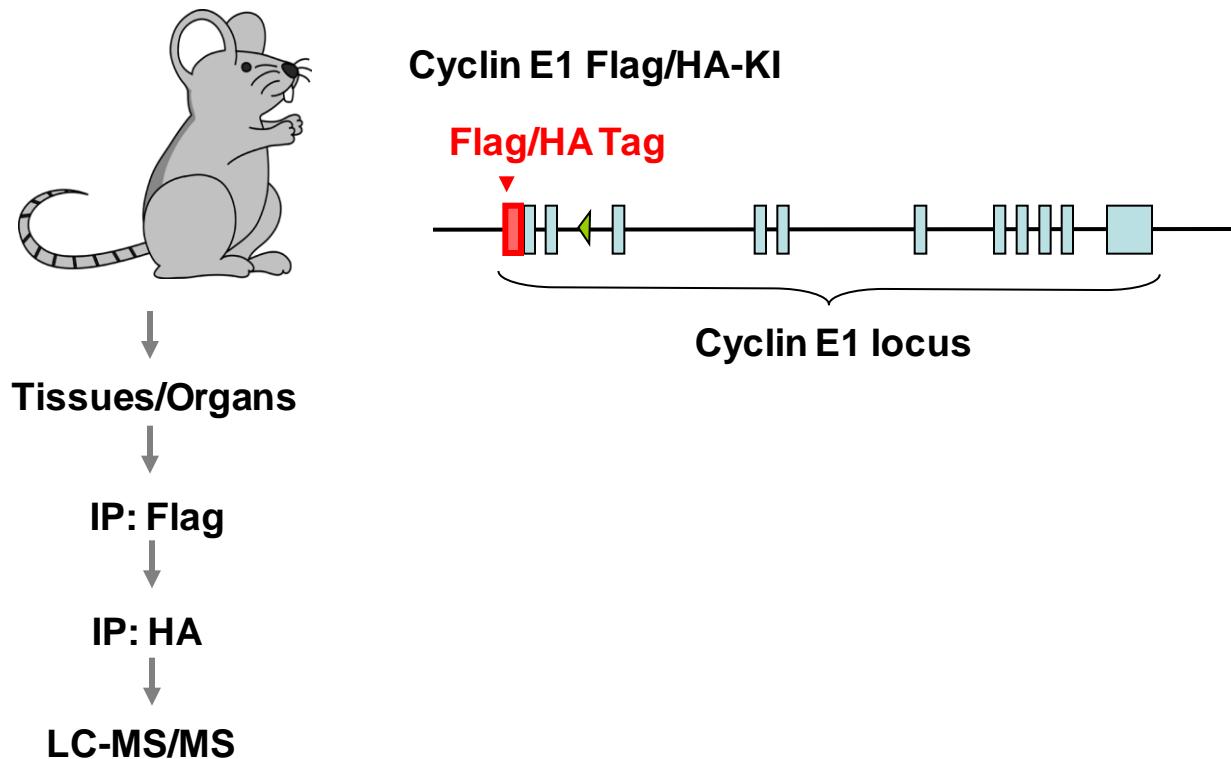
Mass Spectrometry

Cyclin E1 Flag/HA tagging

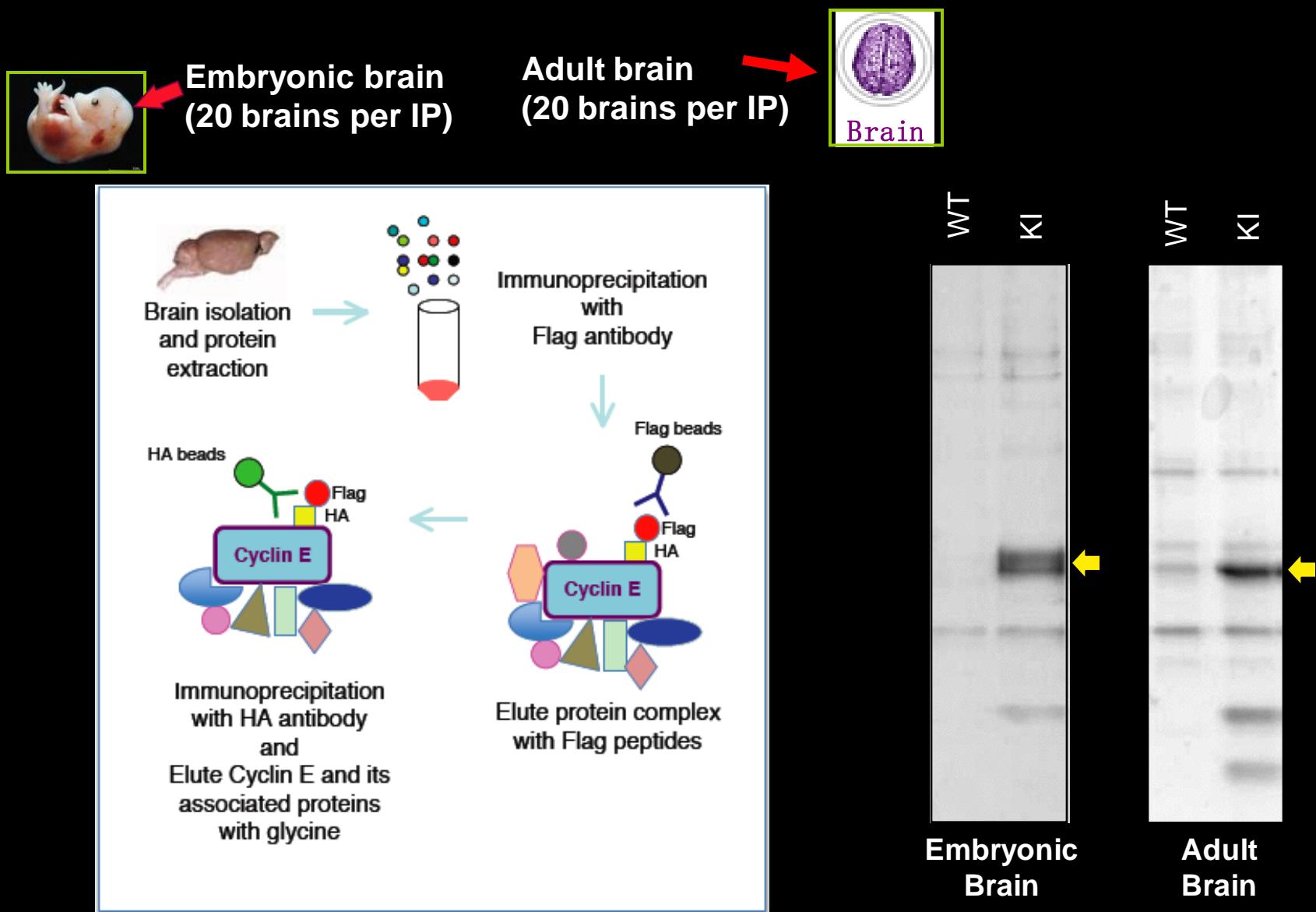
Cyclin E1 NTAG-KI



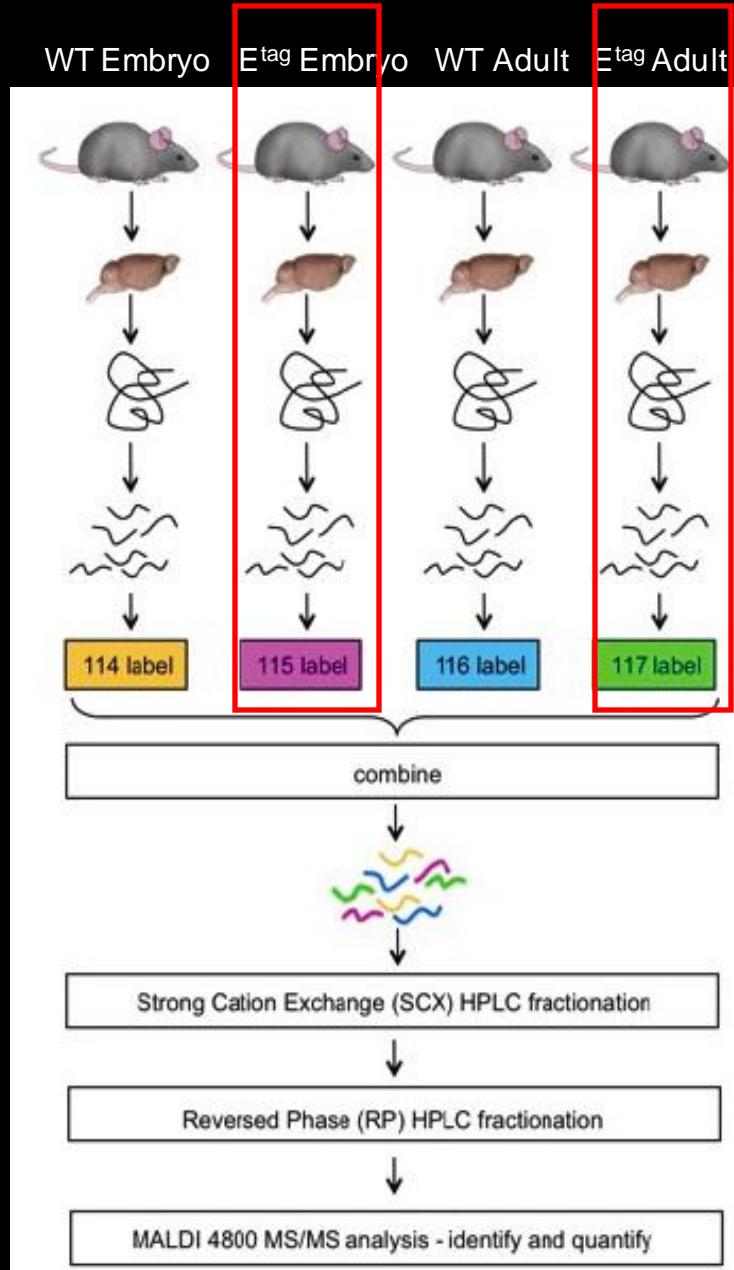
Generation cyclin E1^{tag/tag} mice



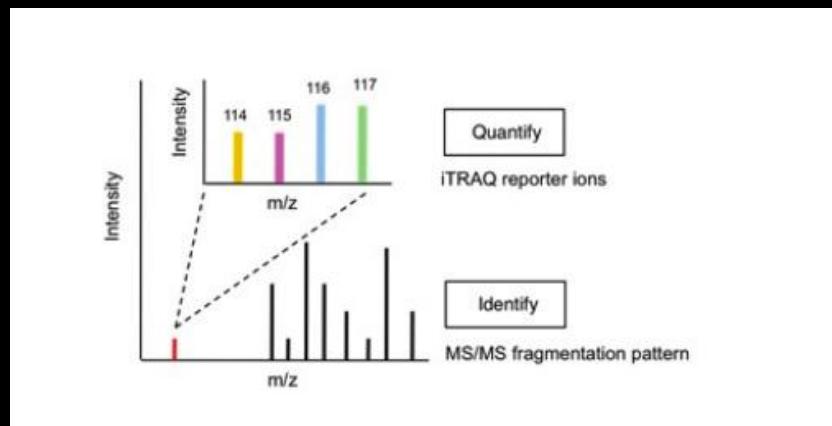
Purification of cyclin E1-associated proteins



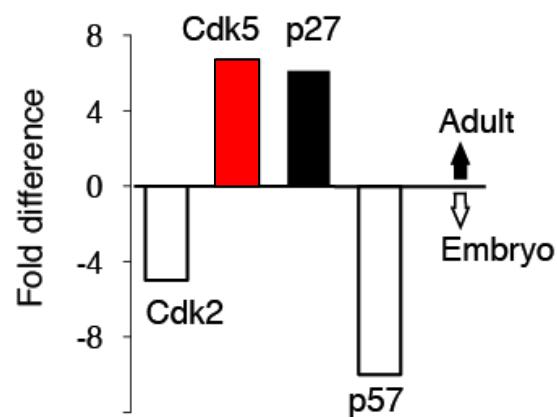
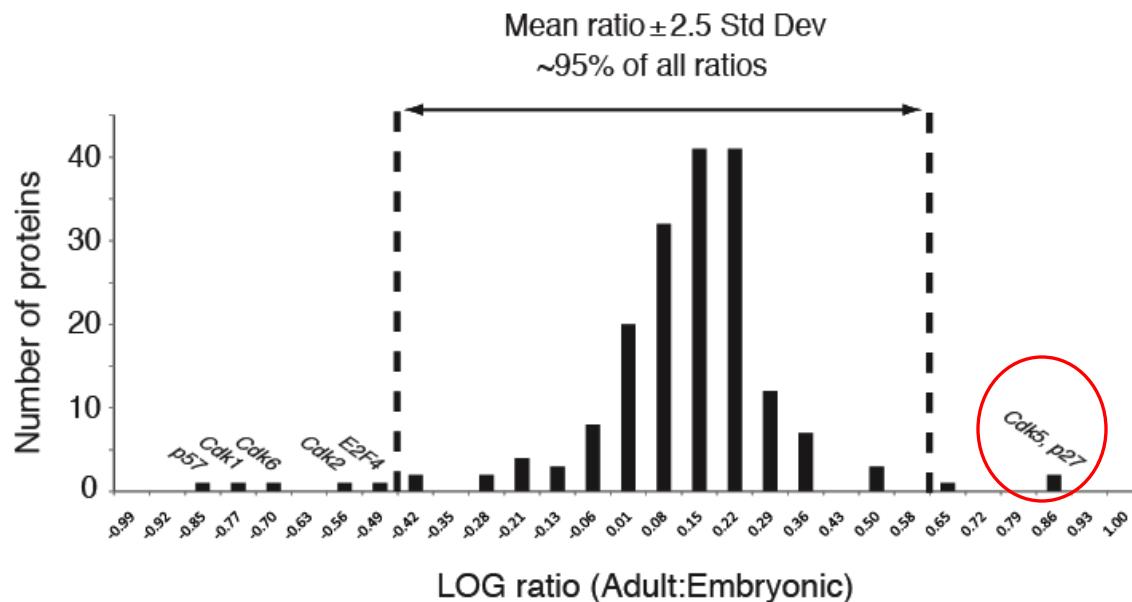
iTRAQ Analysis



1. Brain isolation
2. Protein extraction and purification
3. Trypsin digestion
4. iTRAQ labeling



Embryonic Brain vs Adult Brain

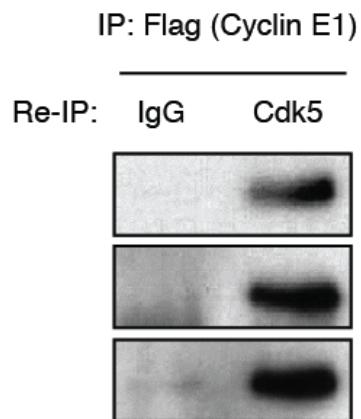
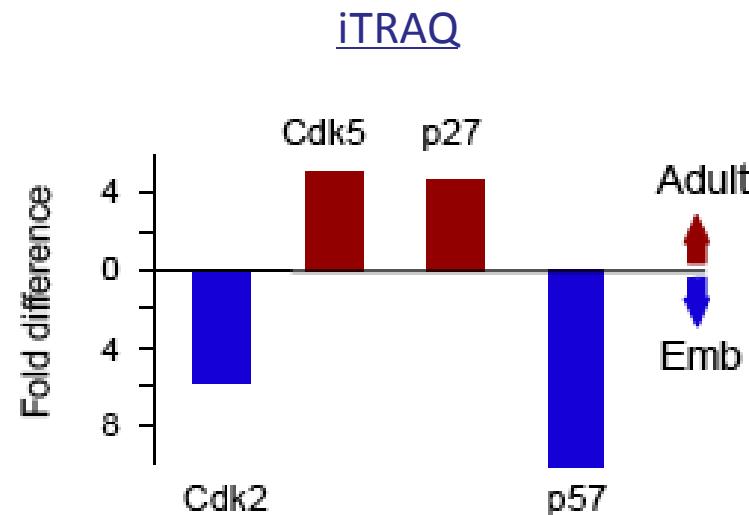
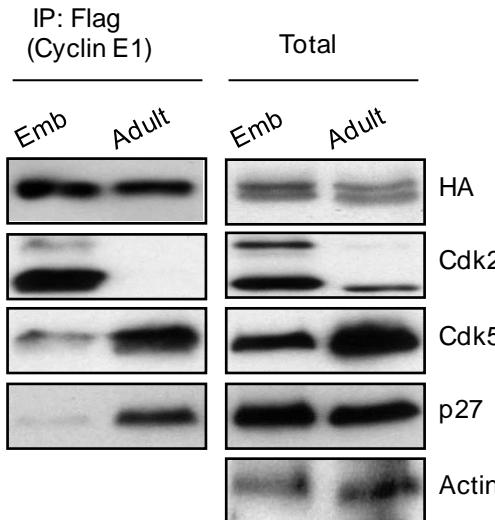


CDK5

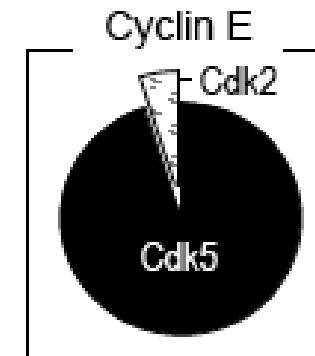
Required for neuronal differentiation

partners with p35 and p39

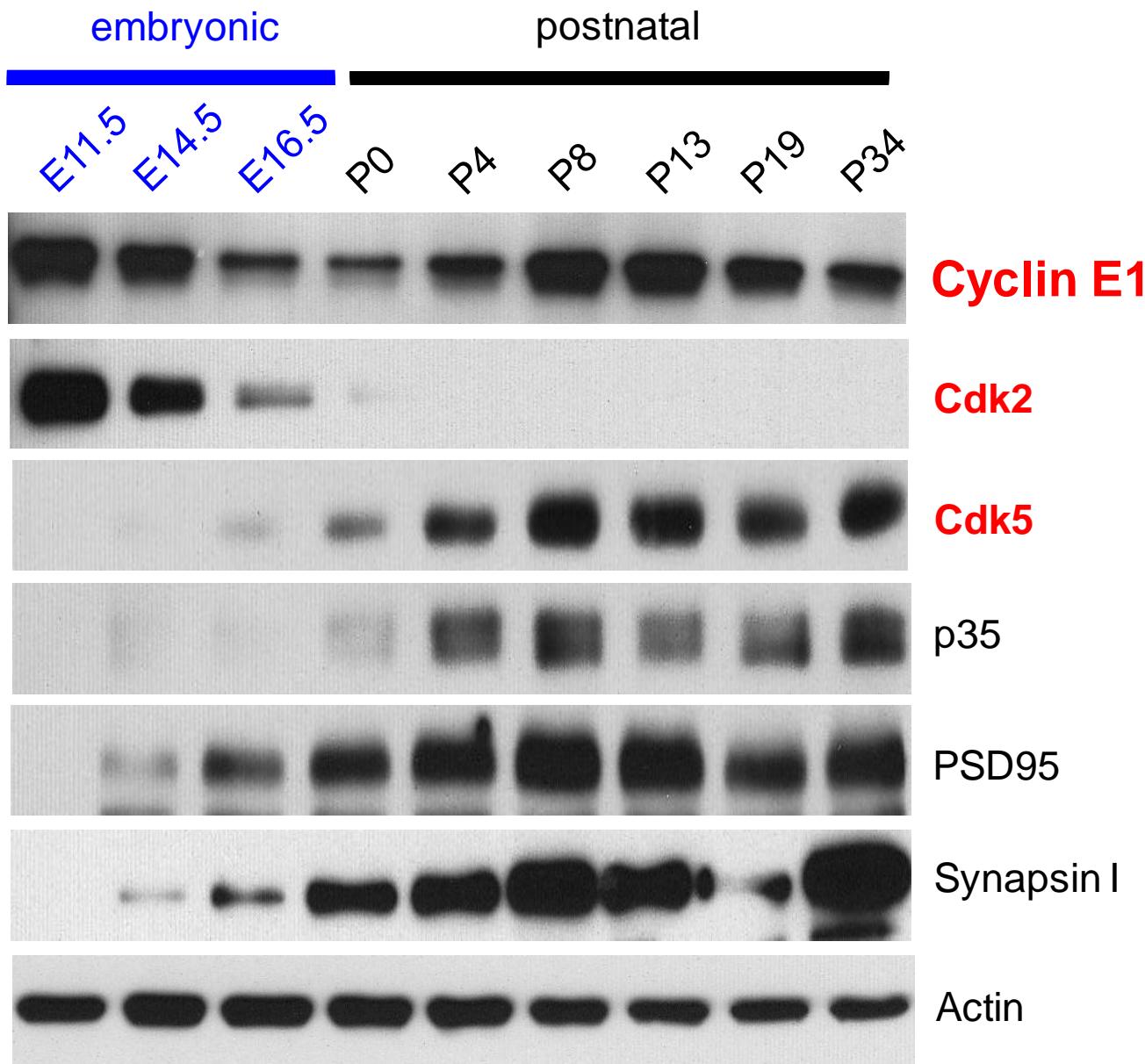
plays key roles in neuronal differentiation



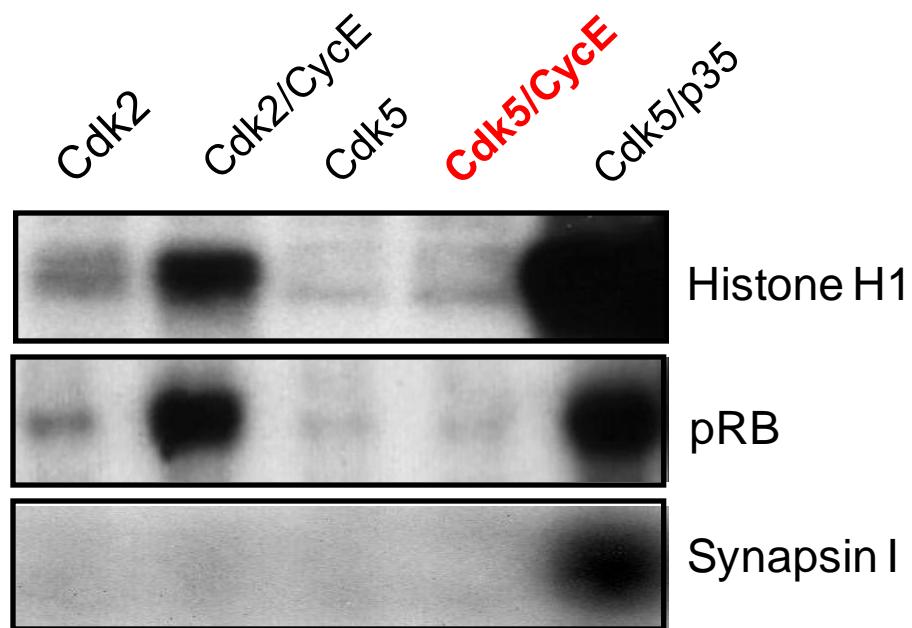
Immunodepletion Experiments



Developmental expression of cyclin E in the brain

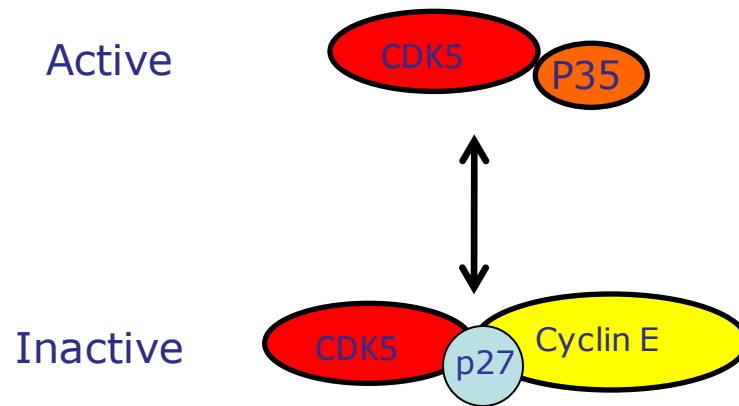


Cyclin E-Cdk5 complexes are catalytically inactive

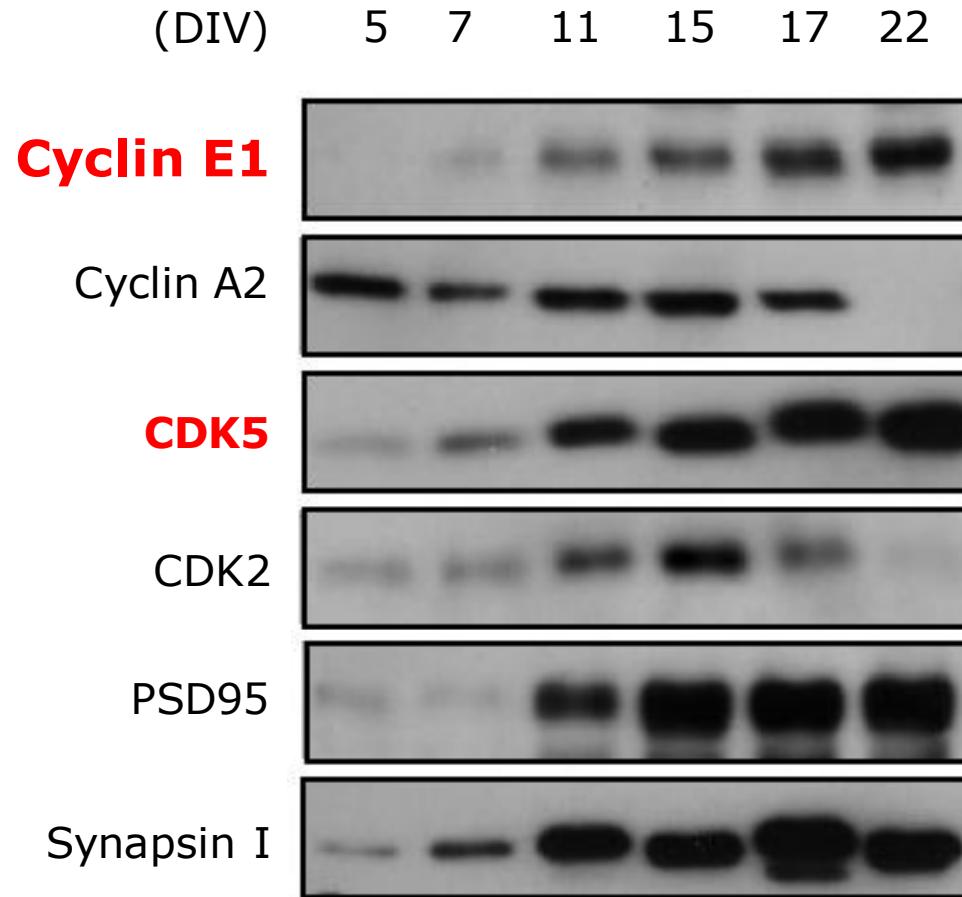


Cyclin E inhibits Cdk5 kinase activity

Two CDK5 complexes

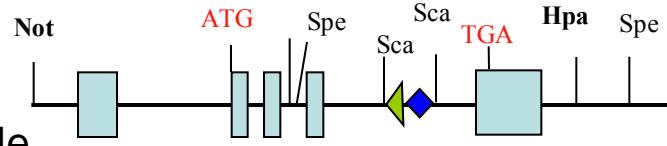
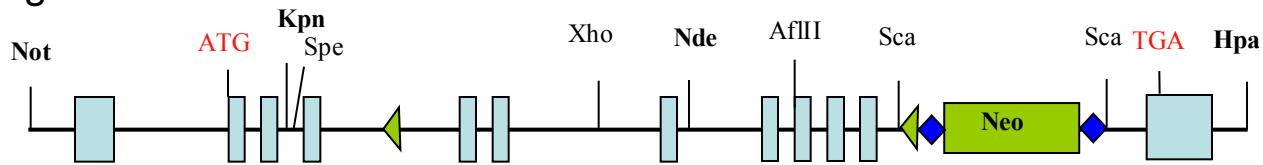


Cyclin E expression during synaptic development



Cyclin E1 conditional KO gene-targeting strategy

Gene-targeting vector



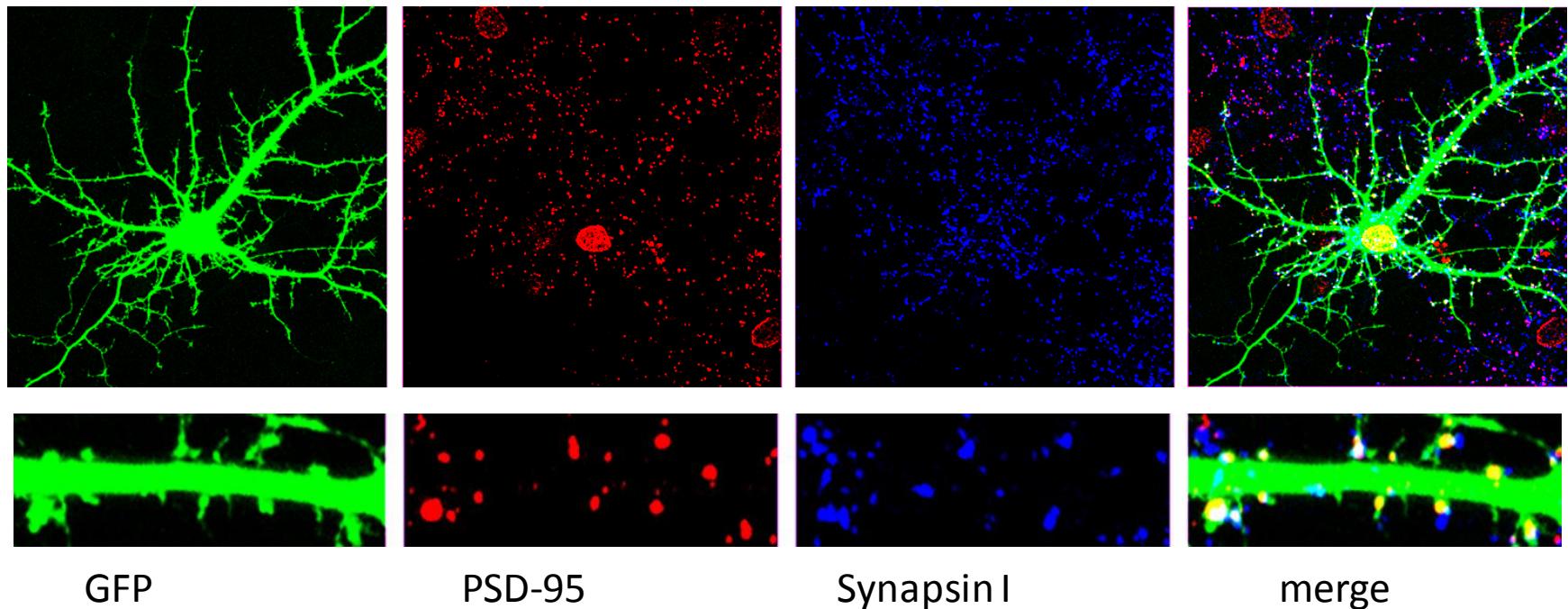
Deleted allele

CyclinE1^{flox/flox}E2^{-/-}

Yan Geng

Assay for synapse development

culture E18 hippocampal neurons → transfect at 7-10 DIV → fix & stain at 14 DIV



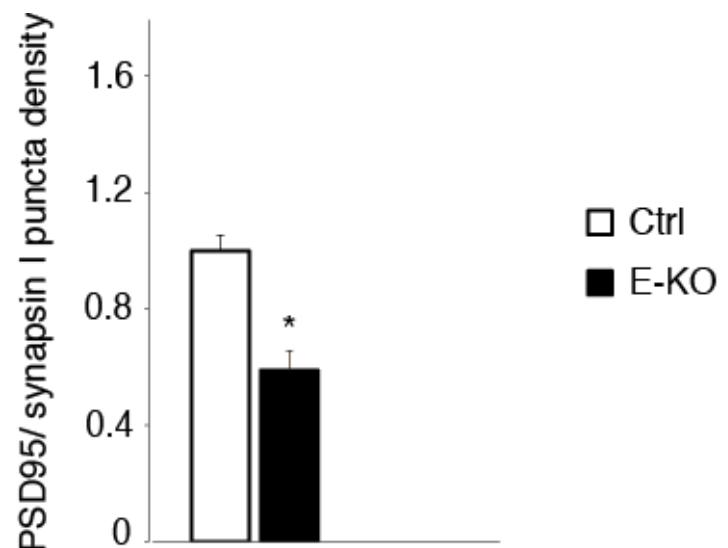
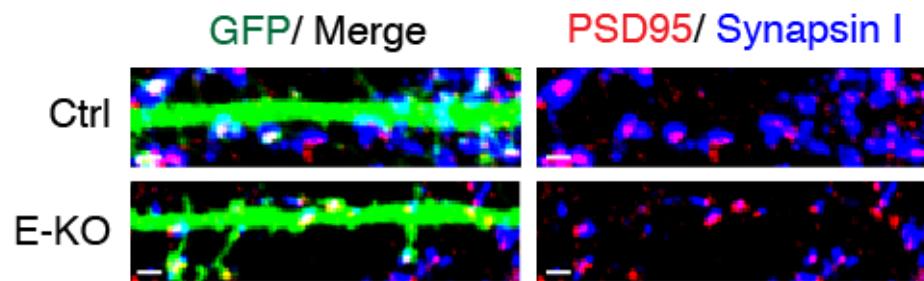
co-localized Synapsin/PSD-95 puncta

Synapse density =

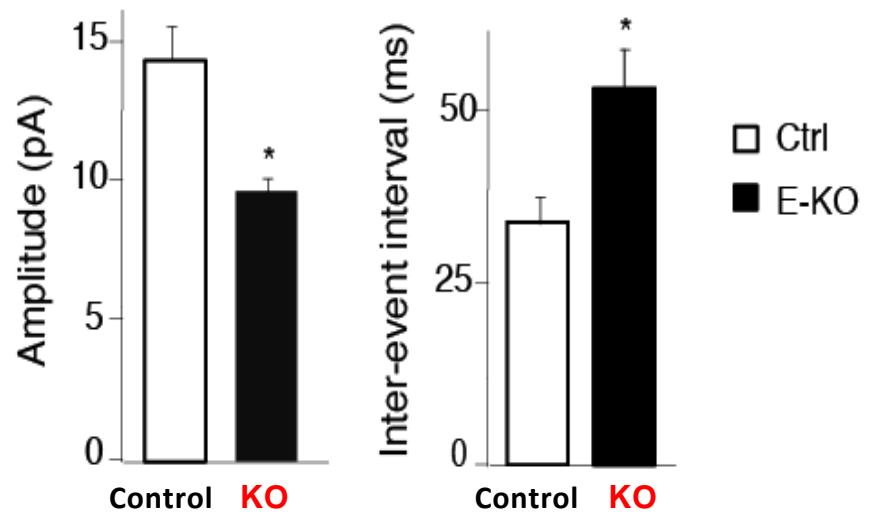
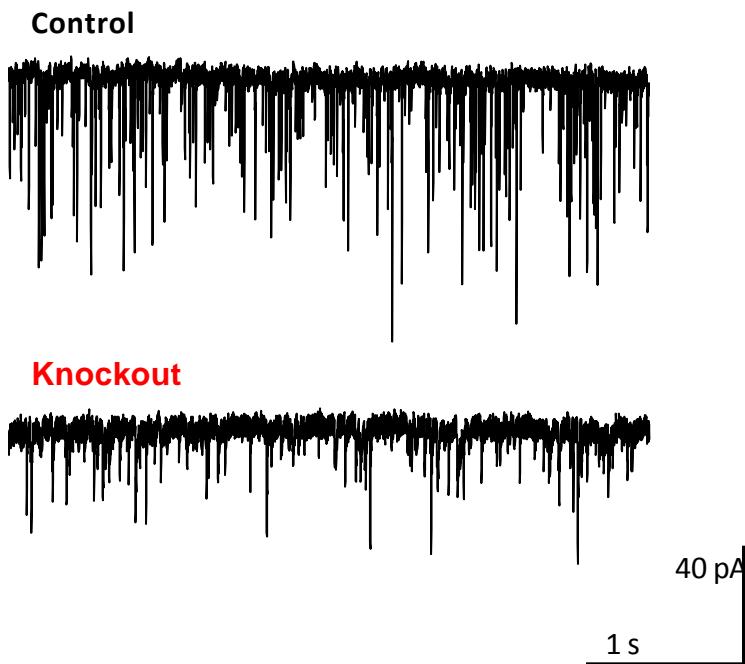
total area of transfected cell

Zak Wills

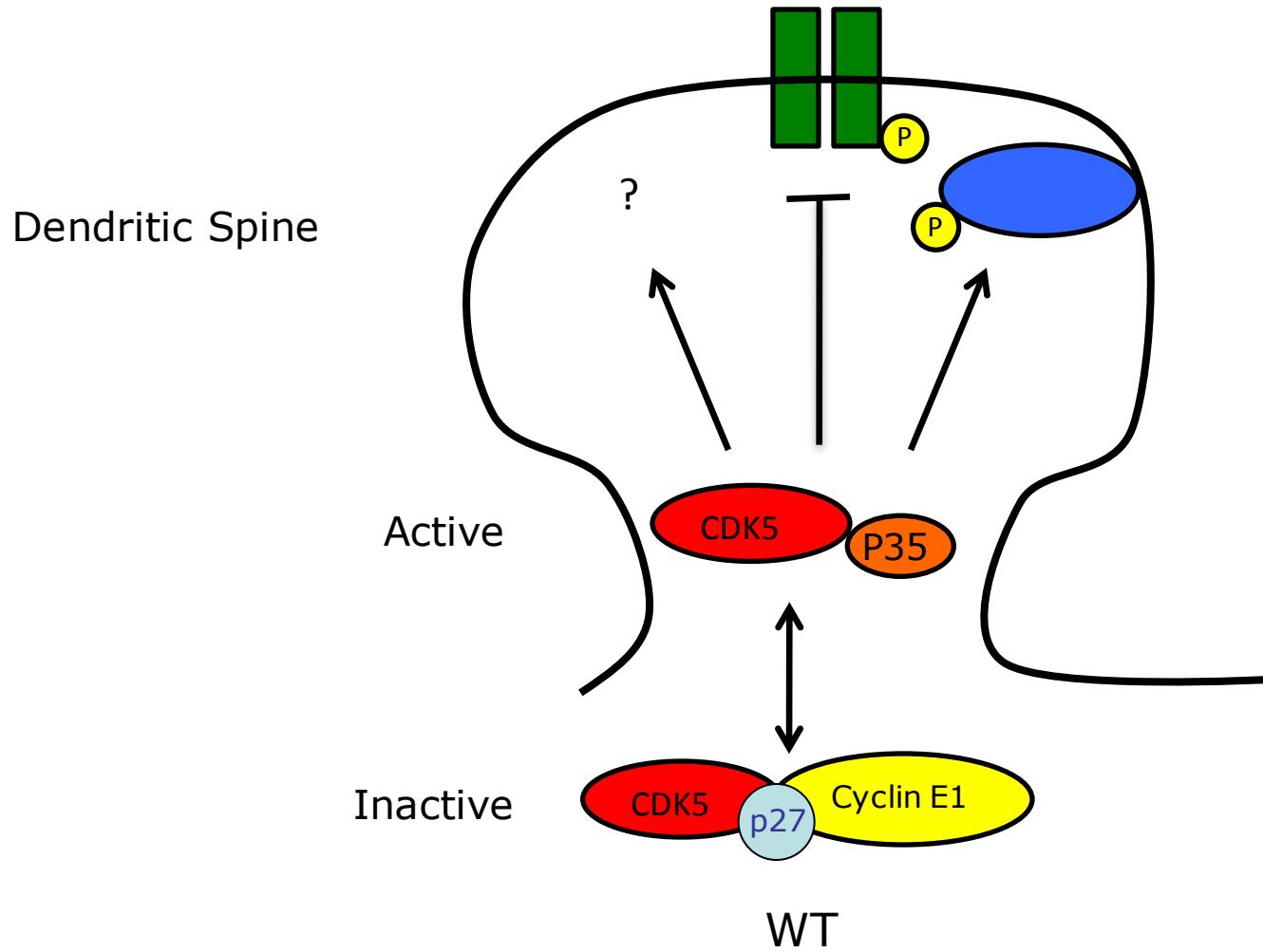
Reduced numbers of synapses upon cyclin E shutdown



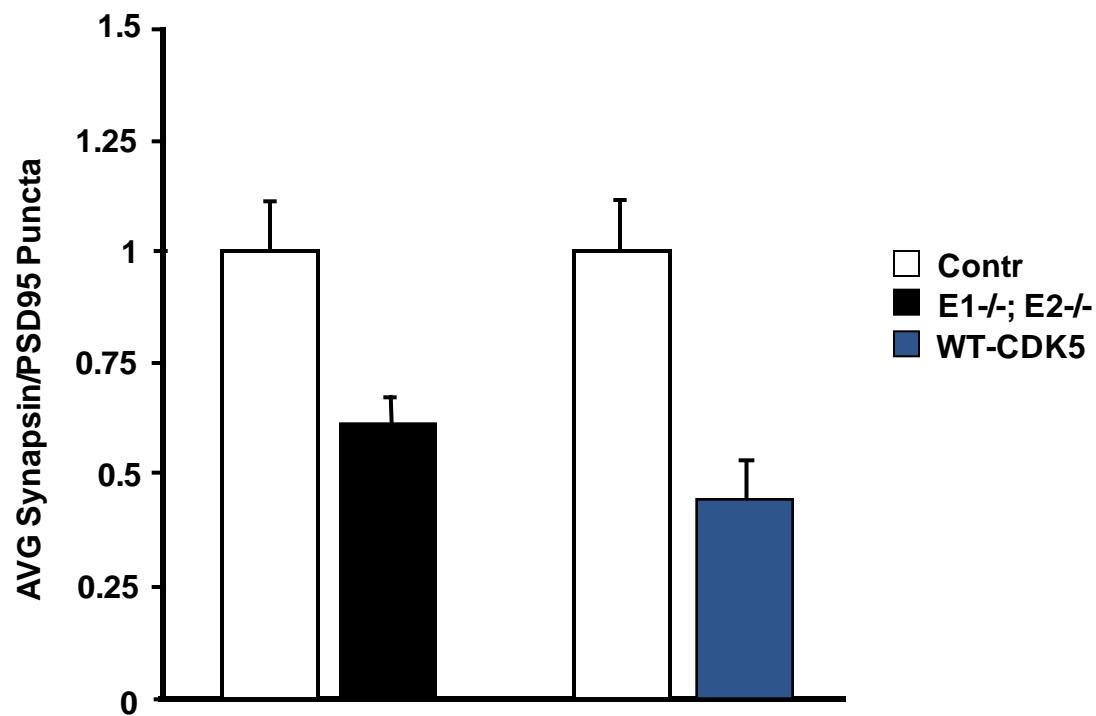
Reduced synaptic currents upon cyclin E shutdown



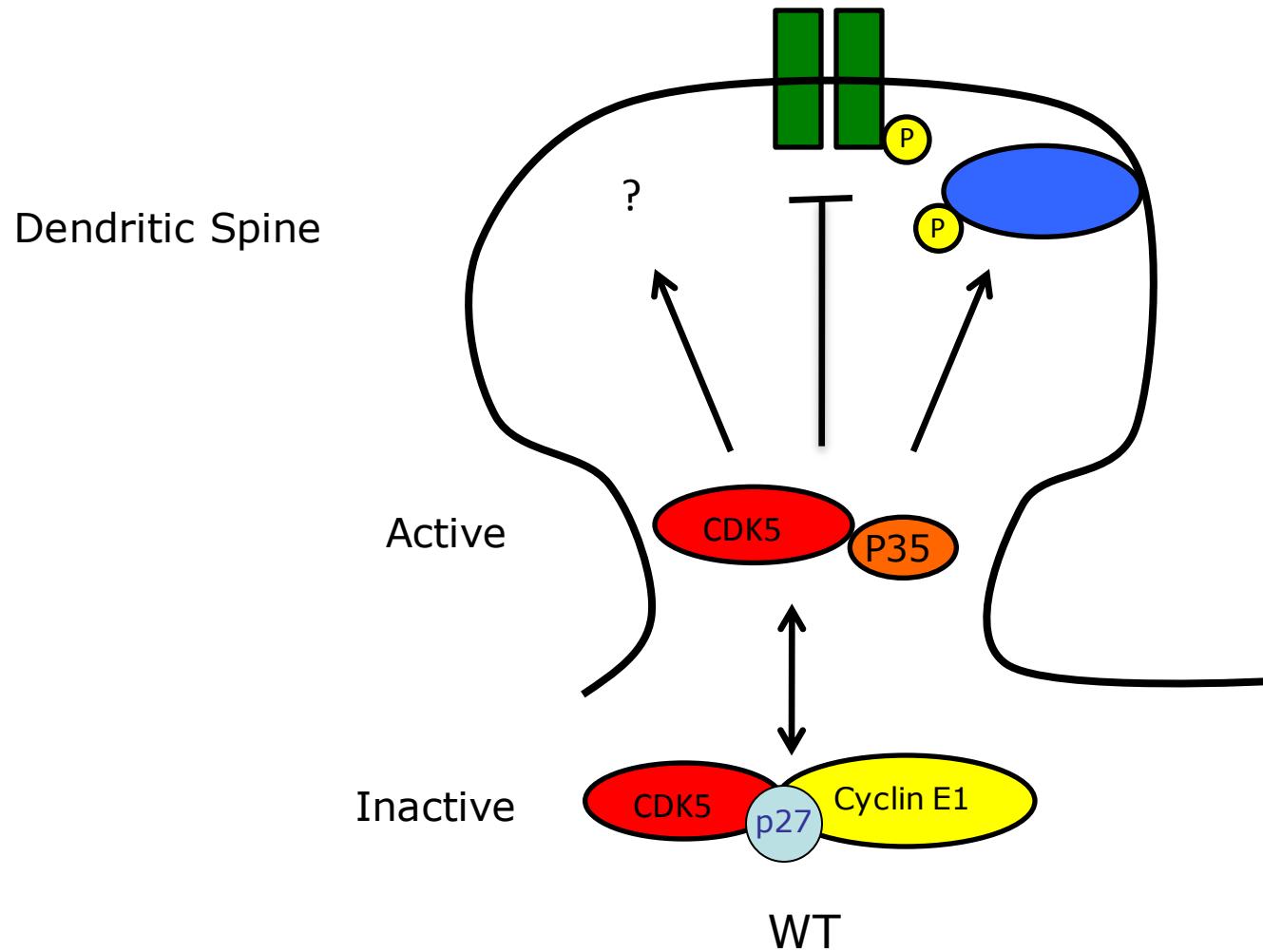
Model how cyclin E regulates CDK5 function



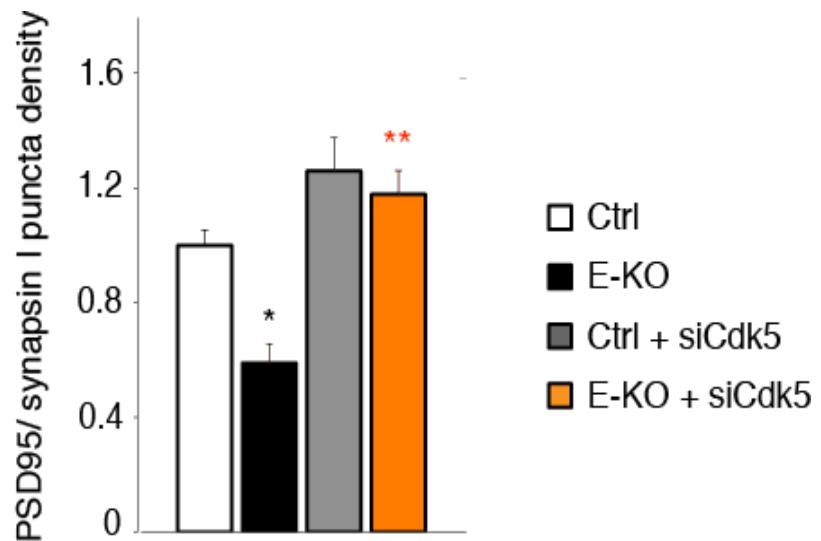
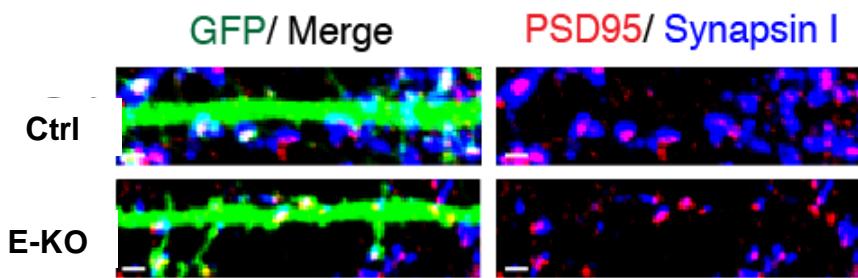
The role of CDK5 in cyclin E-dependent synapse development



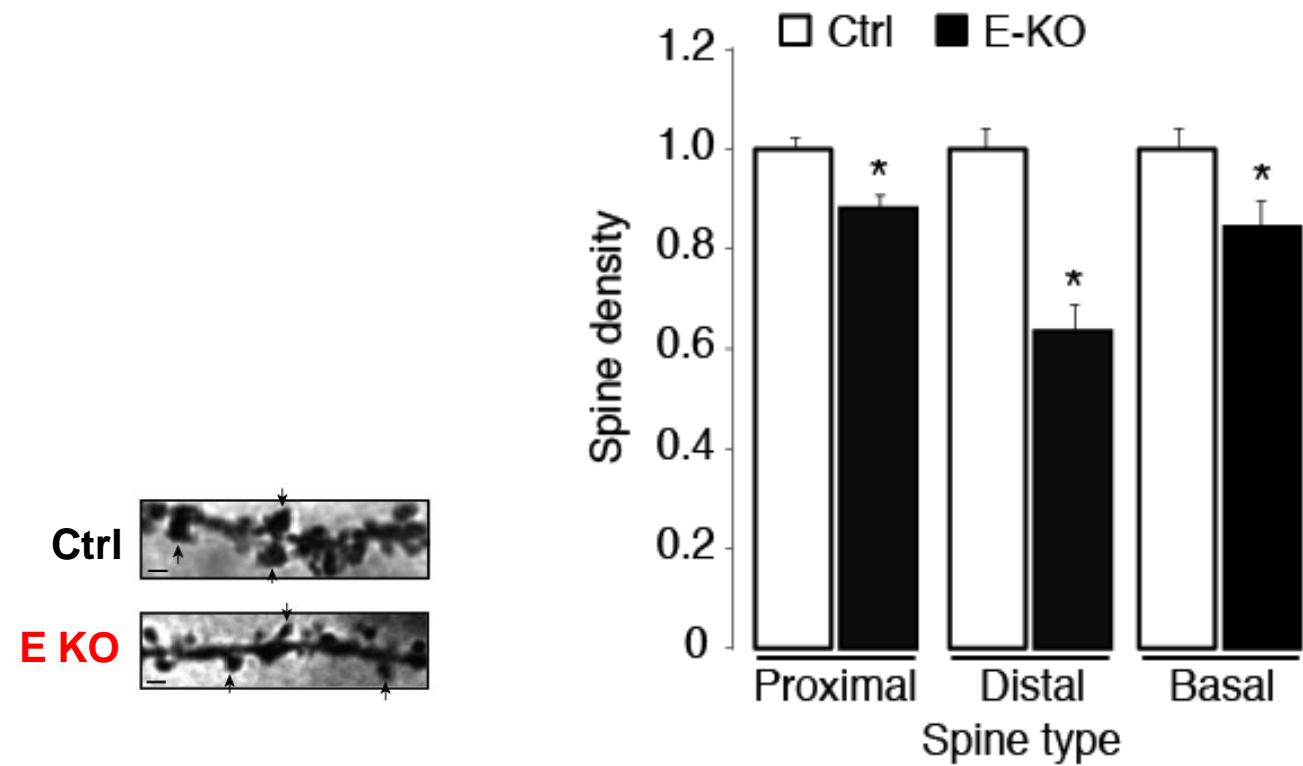
Model how cyclin E regulates CDK5 function



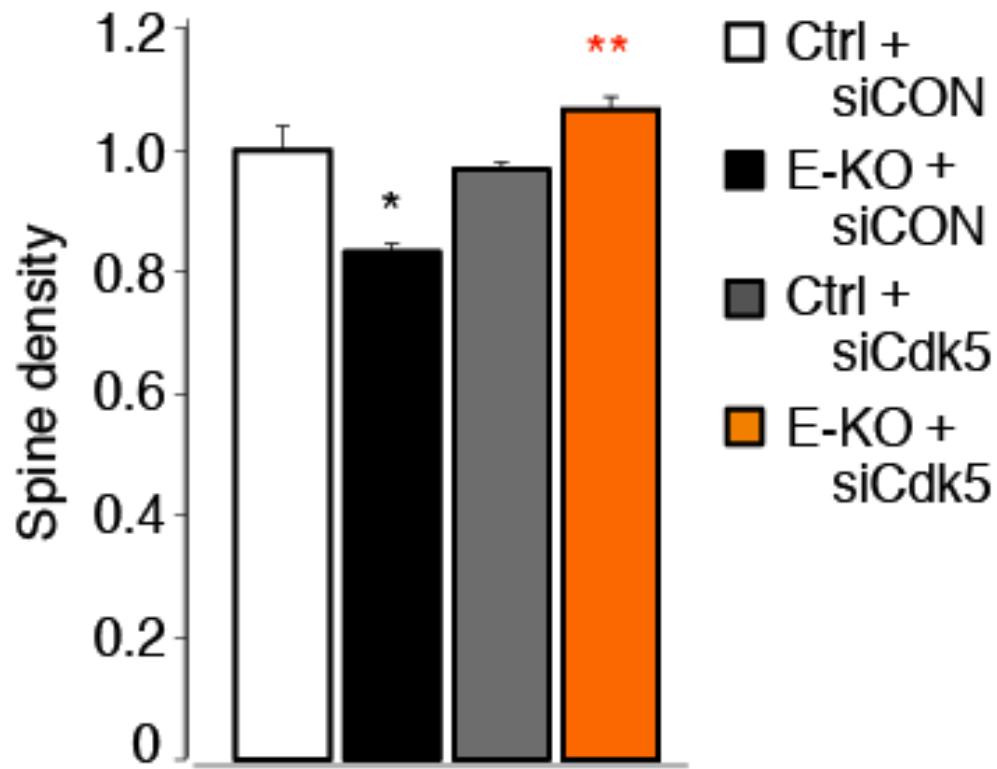
The role of CDK5 in cyclin E-dependent synapse development



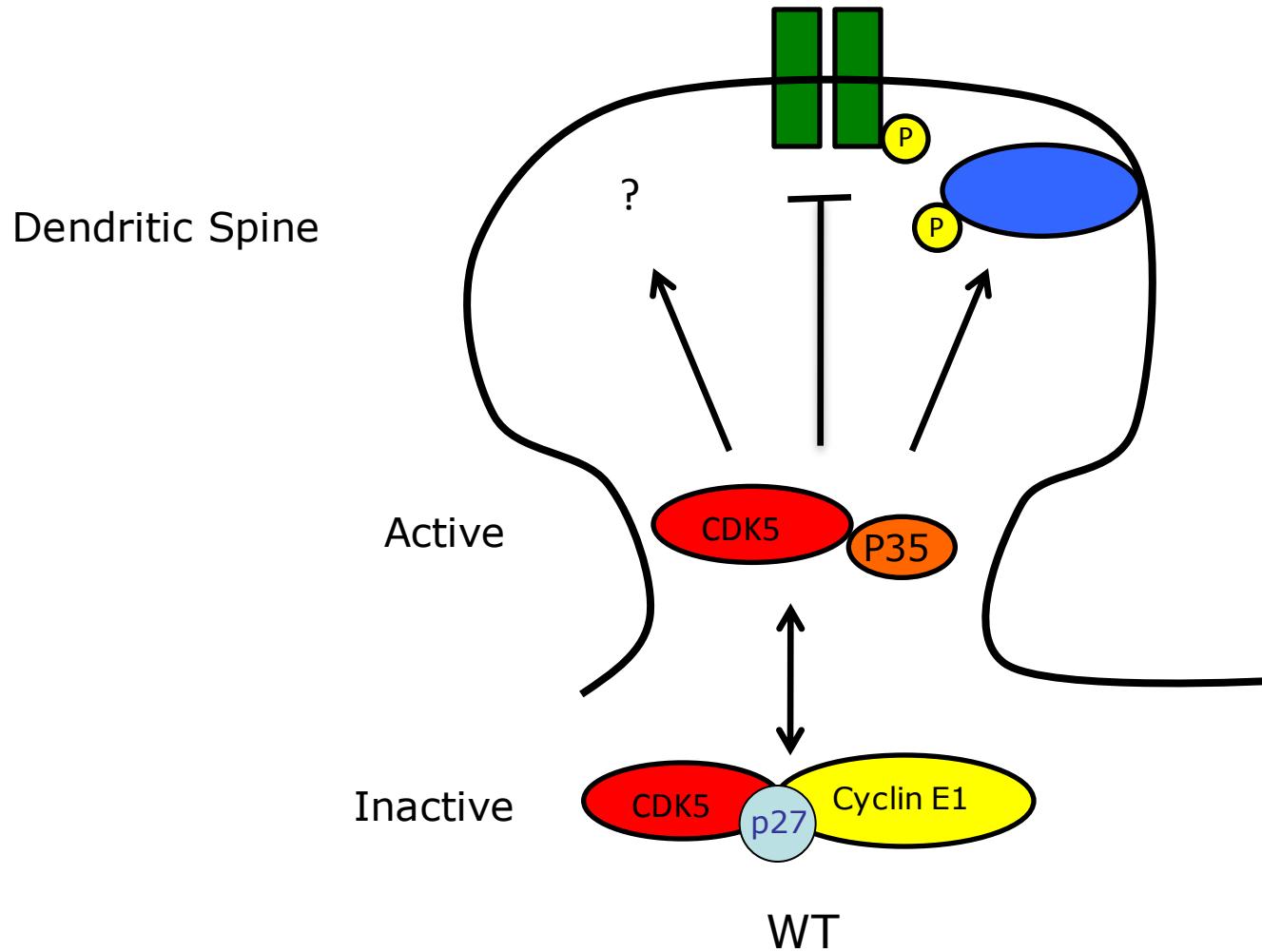
Quantification of dendritic spines *ex vivo* in hippocampal slices



The role of CDK5 in cyclin E-dependent circuit development

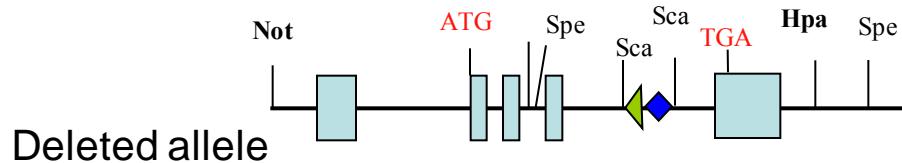
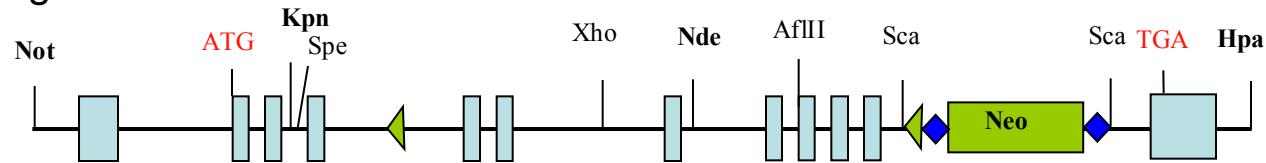


Model how cyclin E regulates CDK5 function



Cyclin E1 conditional KO gene-targeting strategy

Gene-targeting vector



Yan Geng

Cyclin E knockout in the brain

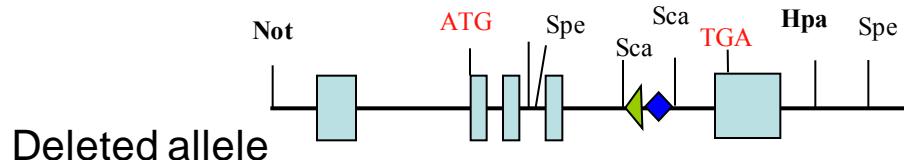
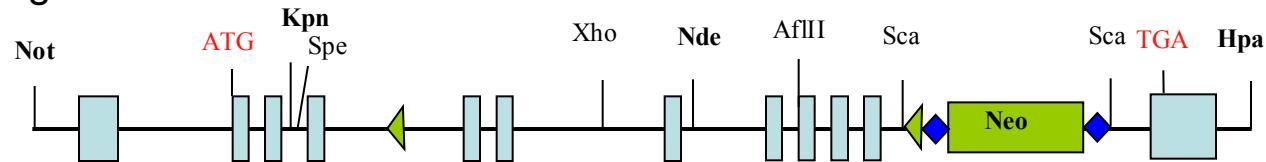
Conditional cyclin E knockout

X

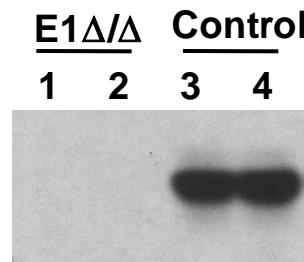
Nestin-Cre

Cyclin E1 conditional KO gene-targeting strategy

Gene-targeting vector



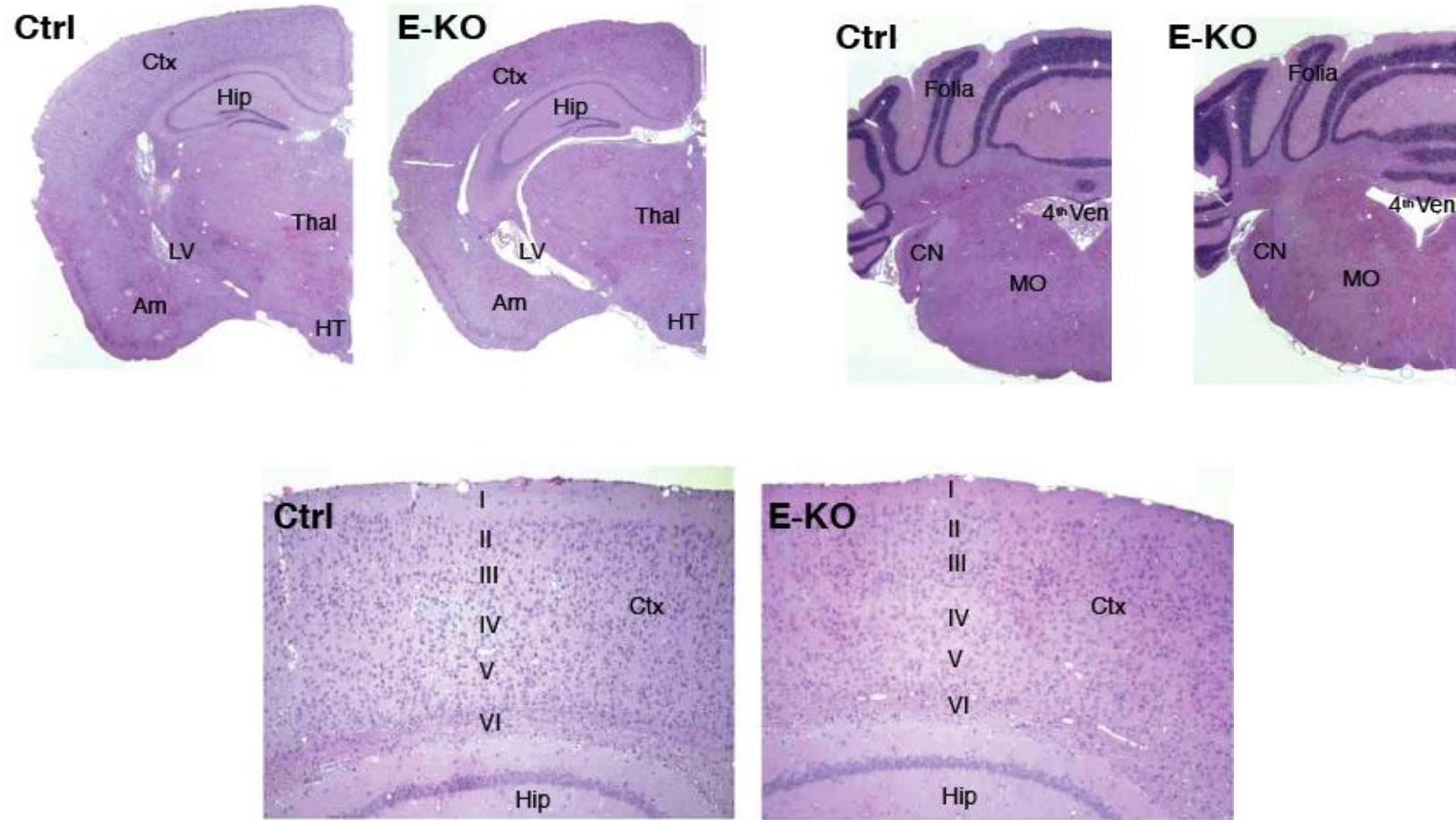
CyclinE1^{flox/flox}E2^{-/-} Nestin-Cre



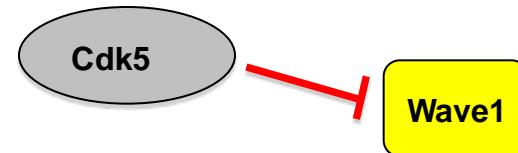
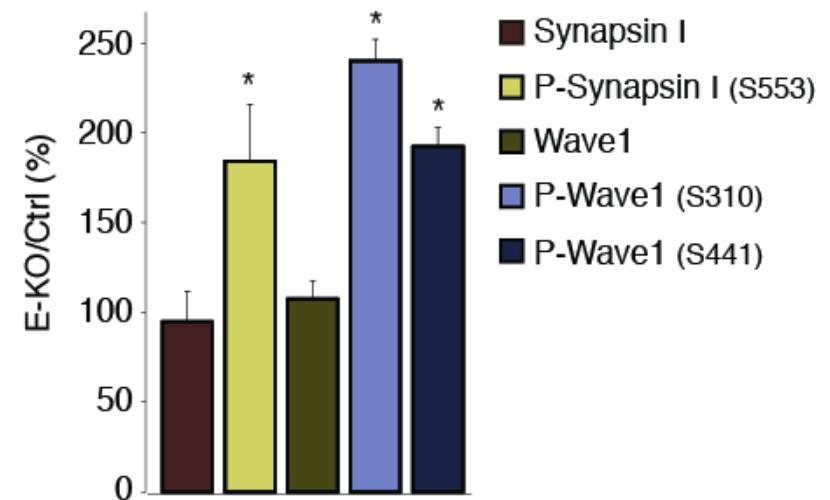
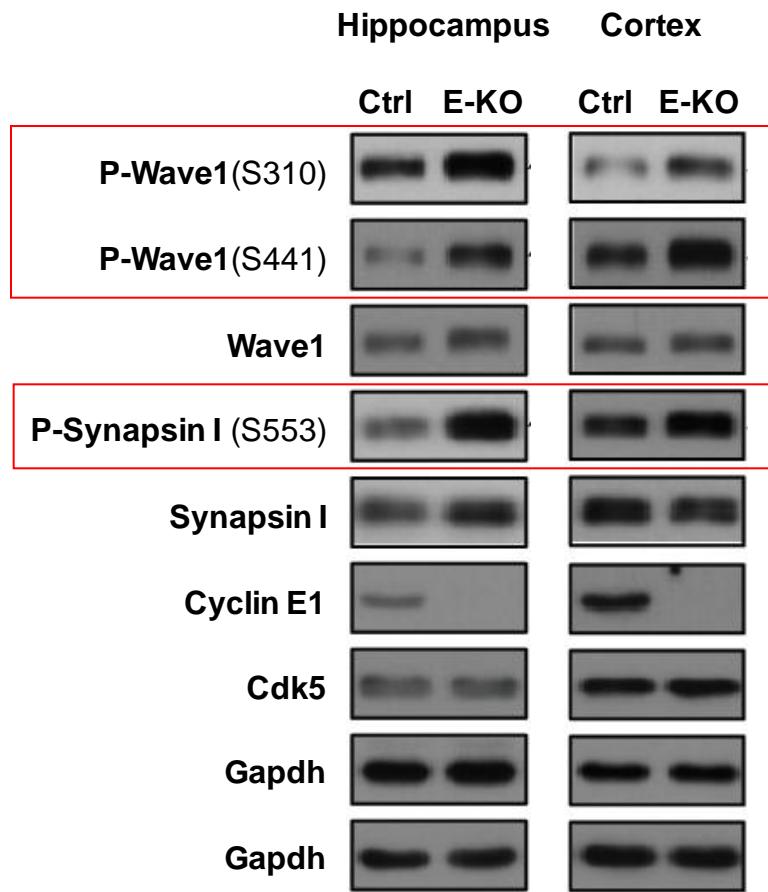
Cyclin E1 levels in 6 week-old brain

Yan Geng

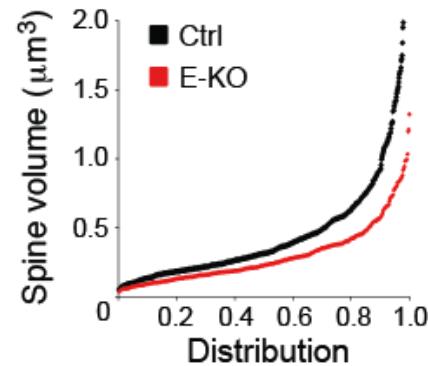
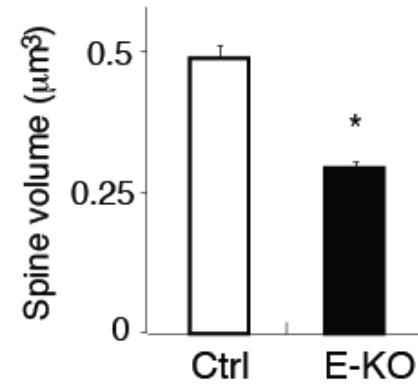
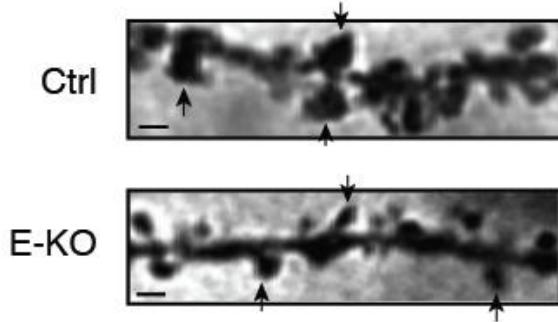
Unperturbed development of cyclin E-null brains



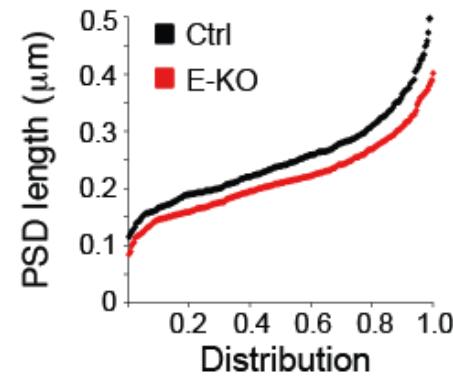
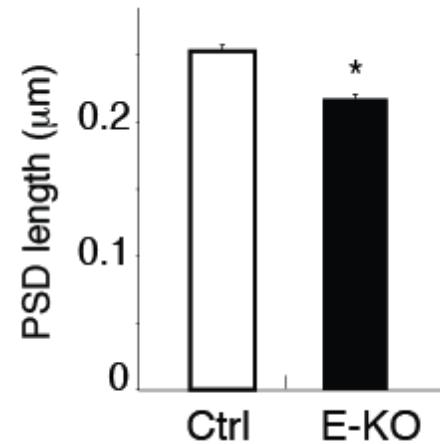
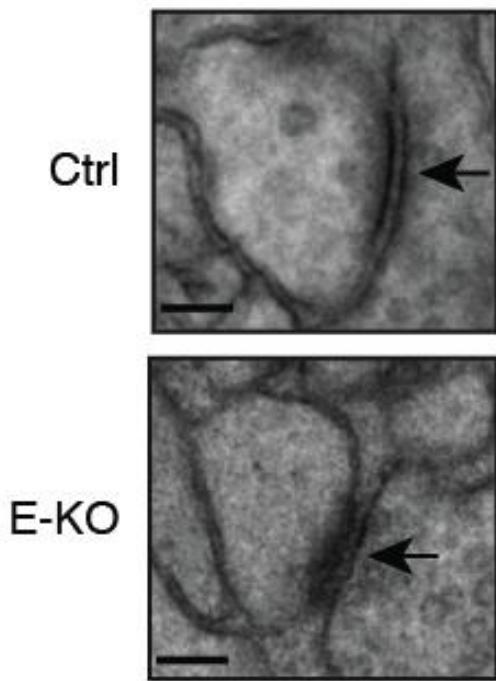
Increased phosphorylation of CDK5 substrates in cyclin E knockout brains



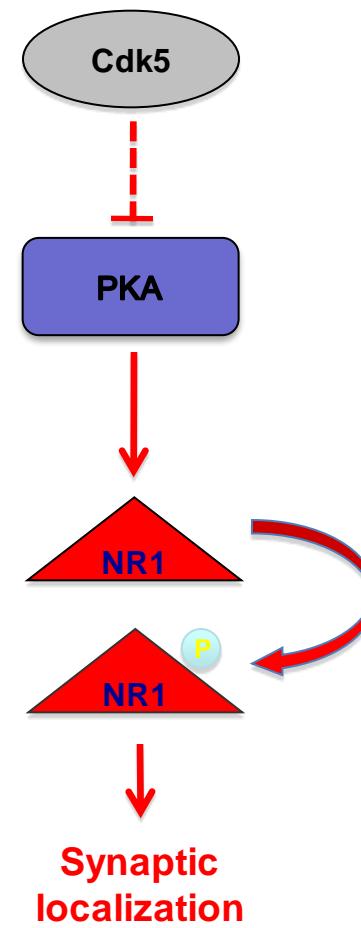
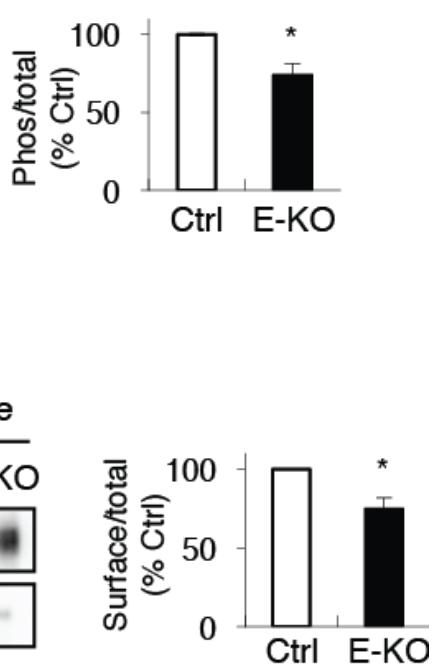
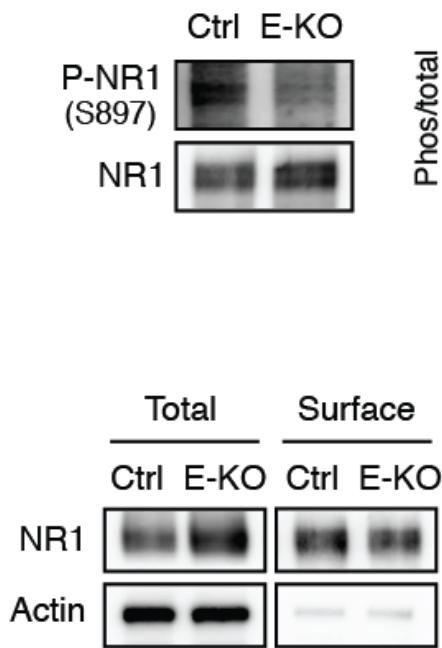
Decreased size of dendritic spines in cyclin E knockout brains



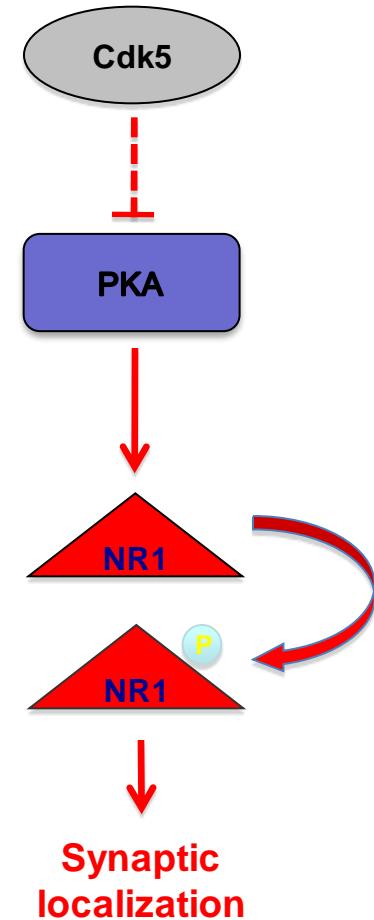
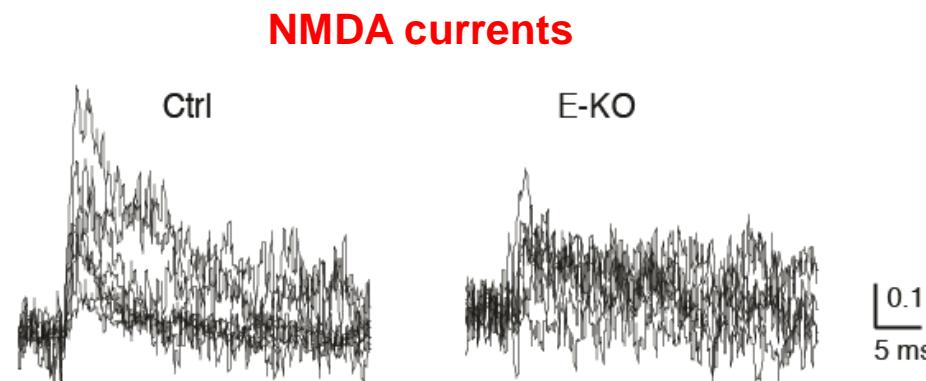
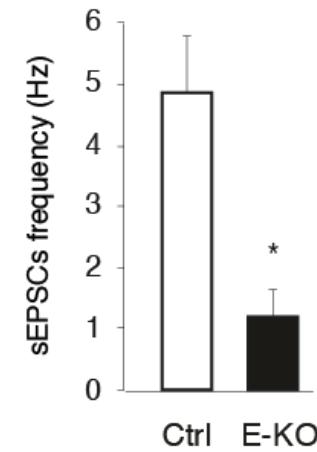
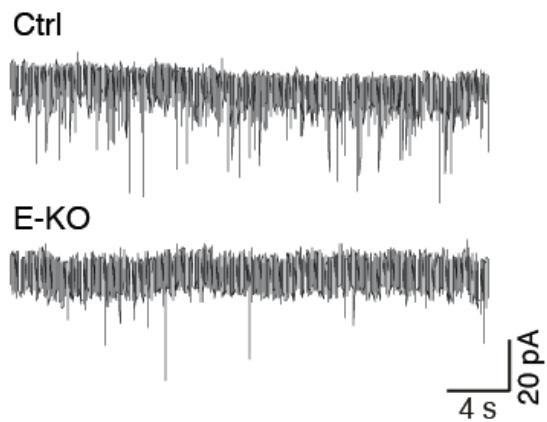
Decreased synapse size in cyclin E knockout brains



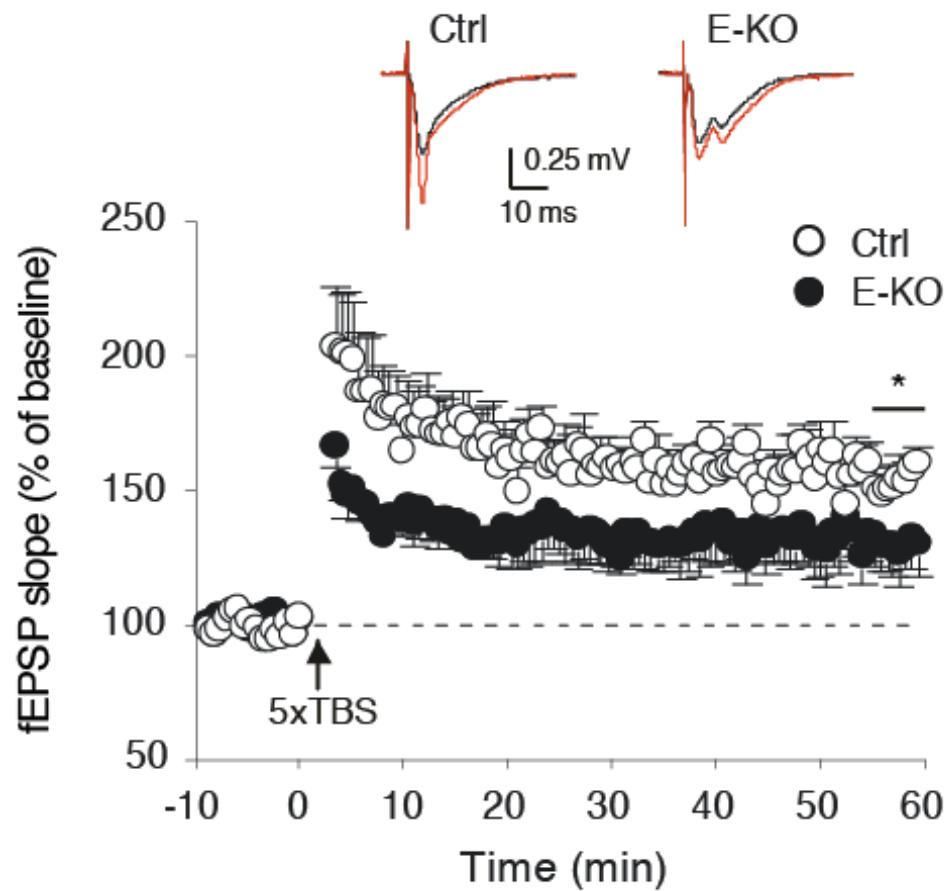
Hypophosphorylation of NR1 (NMDA Receptor) in cyclin E KO



Reduced sEPSP in cyclin E knockout brains

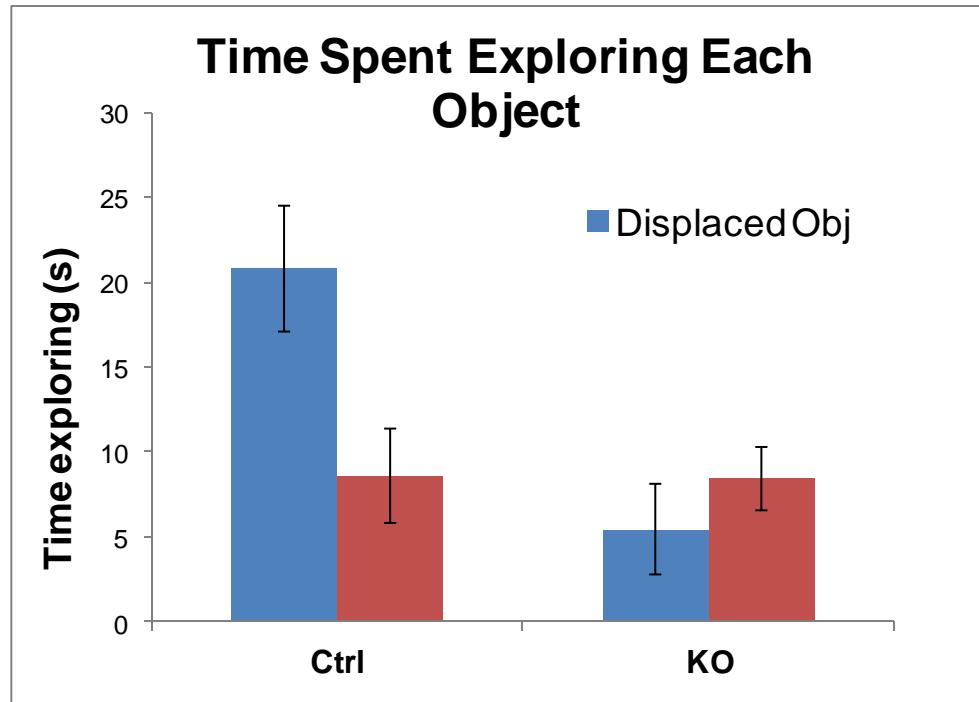
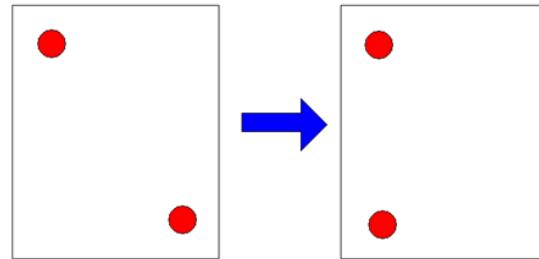


Cyclin E knockout mice display reduced Long-term Potentiation (LTP)



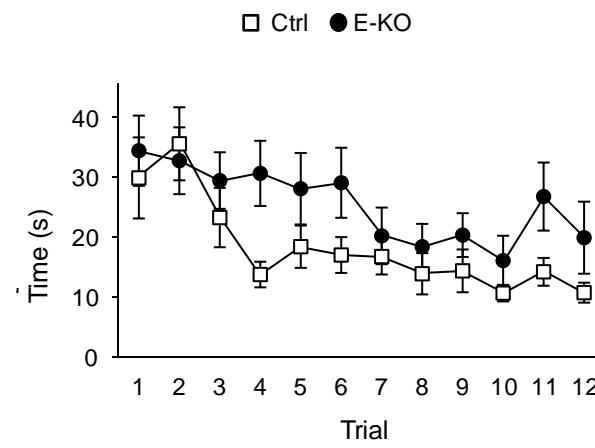
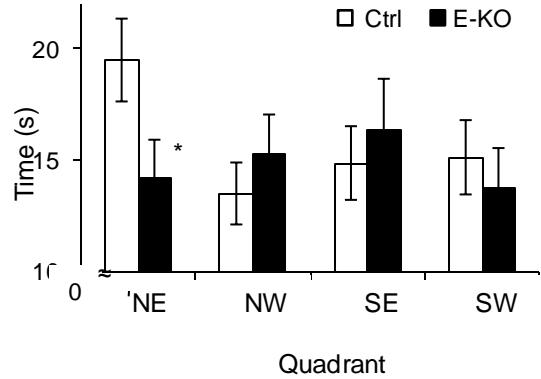
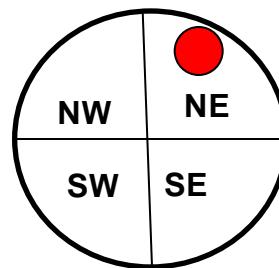
The role of cyclin E in learning and memory

Novel object recognition



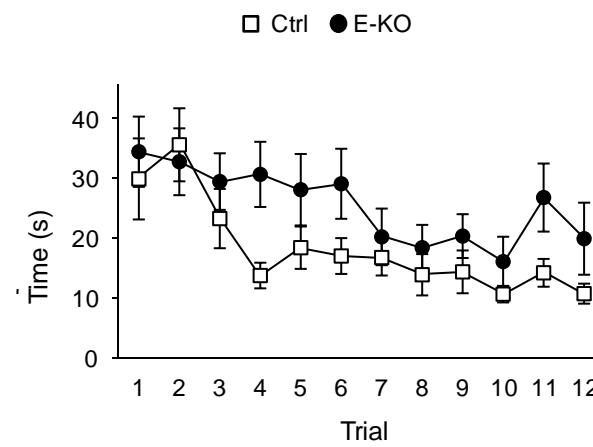
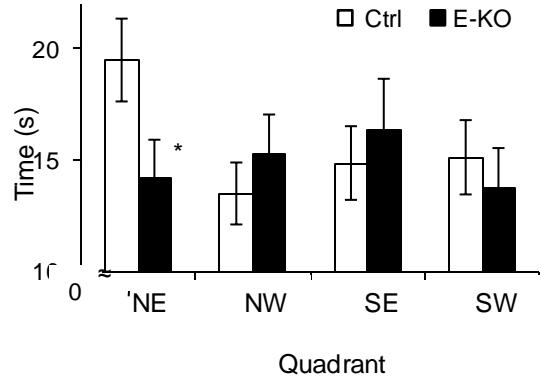
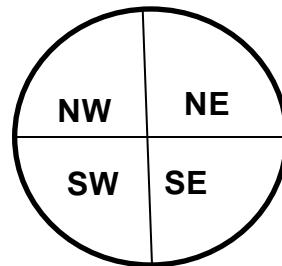
The role of cyclin E in learning and memory

Morris water maze test



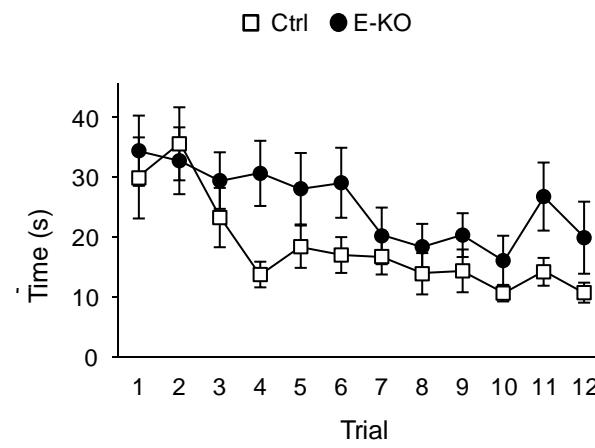
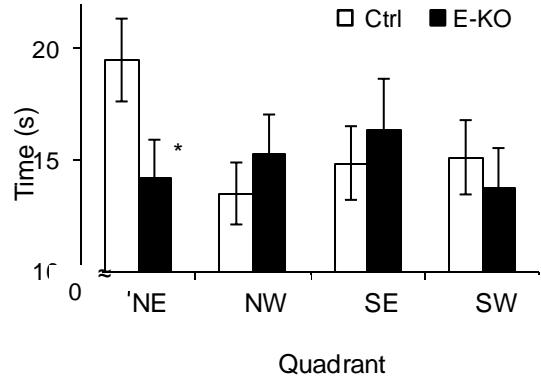
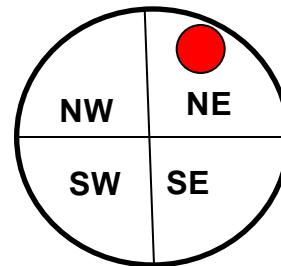
The role of cyclin E in learning and memory

Morris water maze test



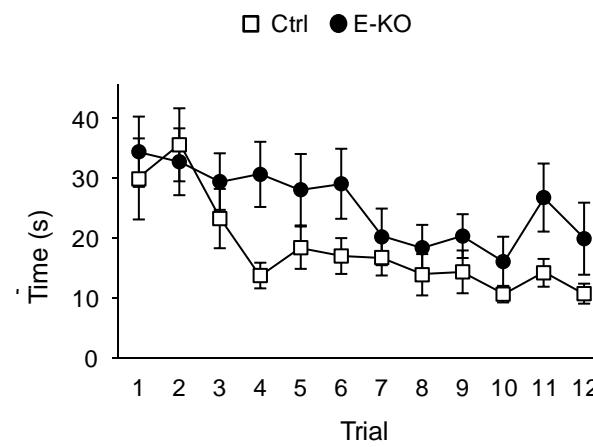
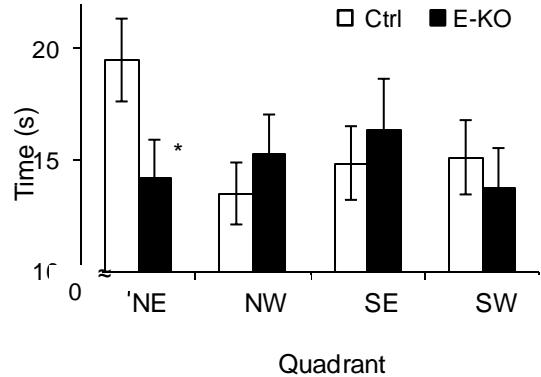
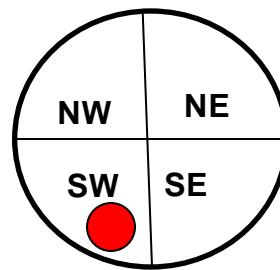
The role of cyclin E in learning and memory

Morris water maze test

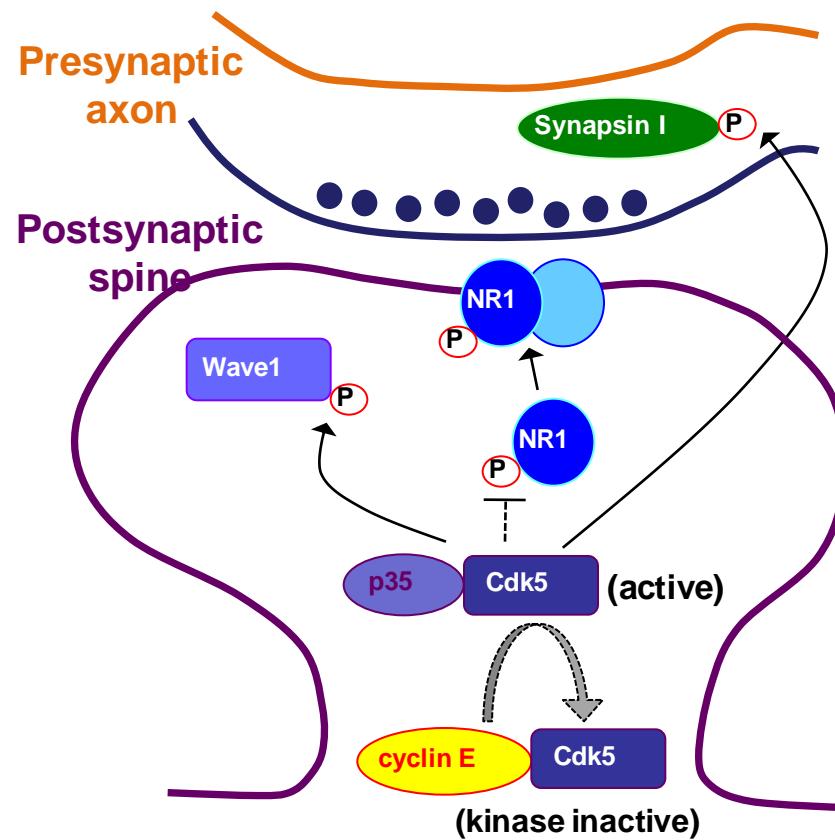


The role of cyclin E in learning and memory

Morris water maze test



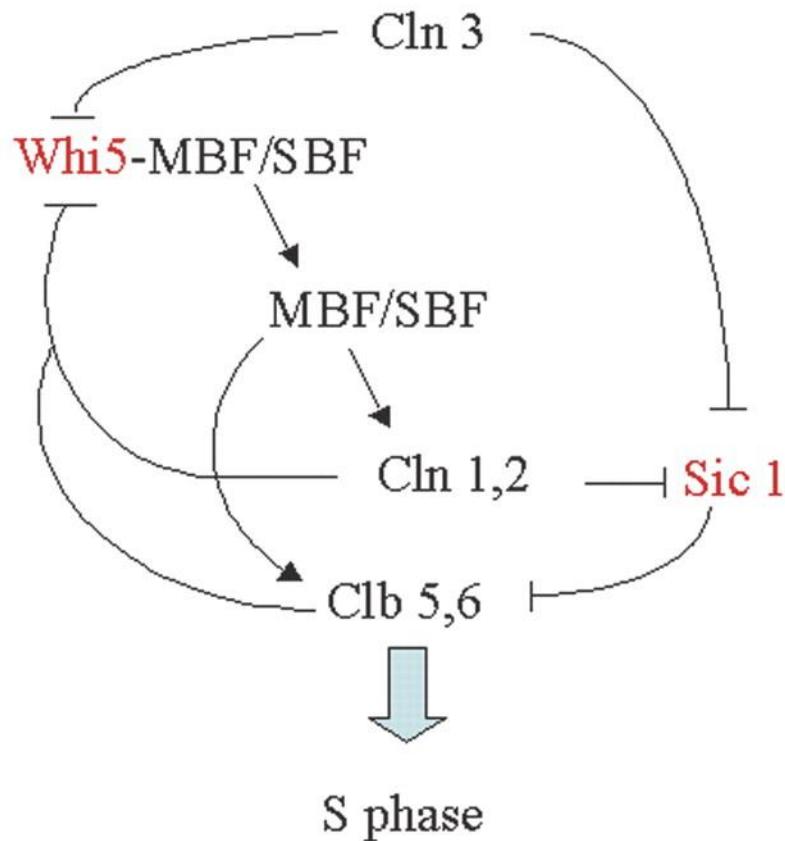
Model how cyclin E regulates CDK5 function



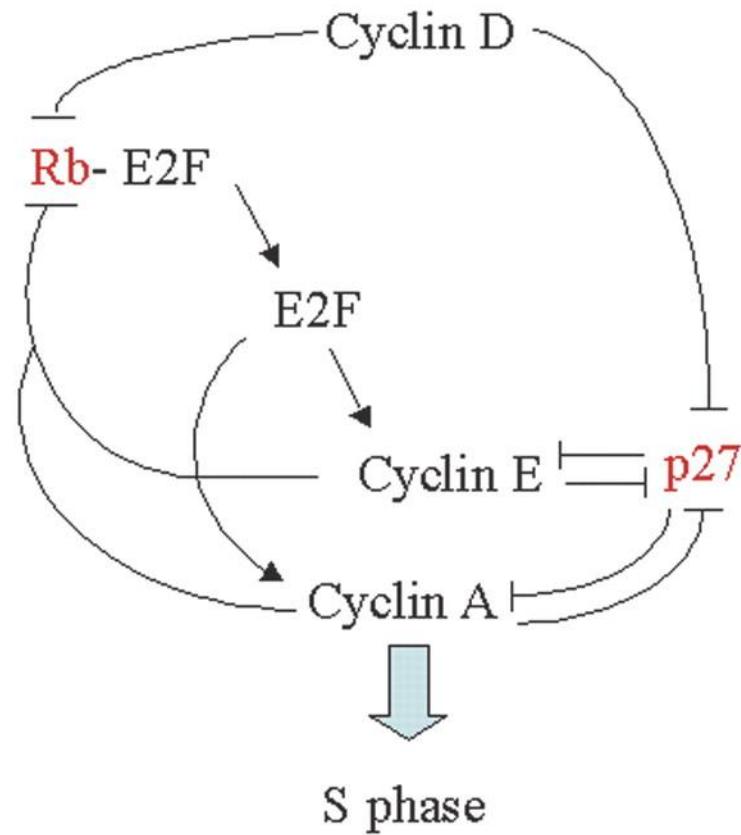


Parallels among the pathways that regulate G1 in budding yeast and mammals.

Budding Yeast



Mammals



Sherr C J , Roberts J M Genes Dev. 2004;18:2699-2711



Junko Odajima

Yan Geng

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