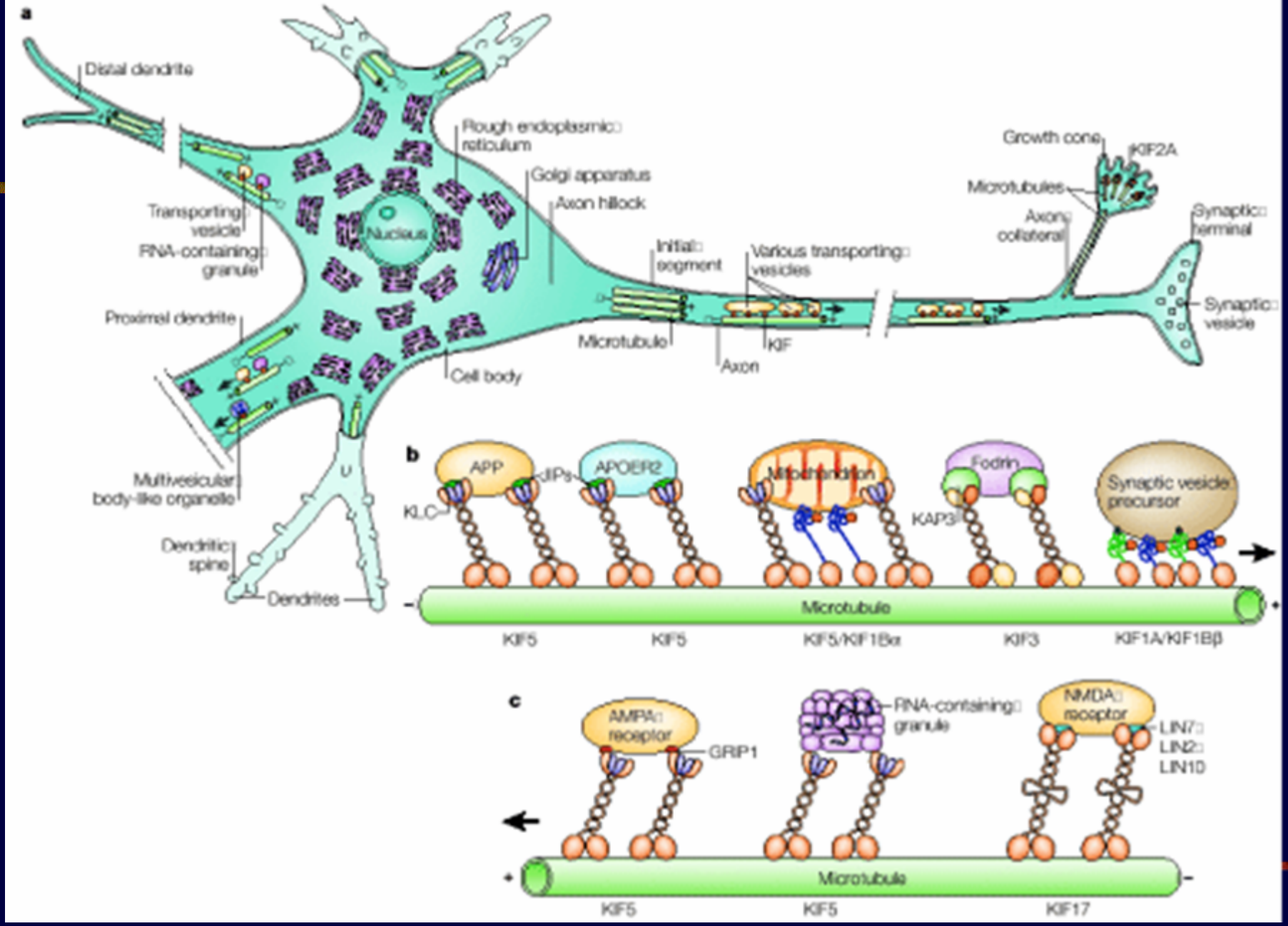
分子モーターから観た生命科学

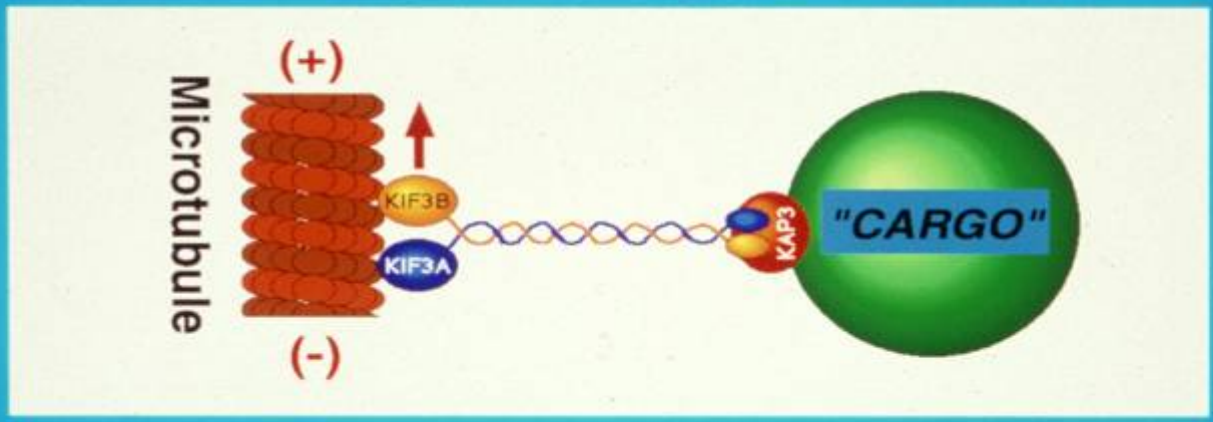
体つくりと分子モーター
分子モーターはどのように動くか

2006年11月27日

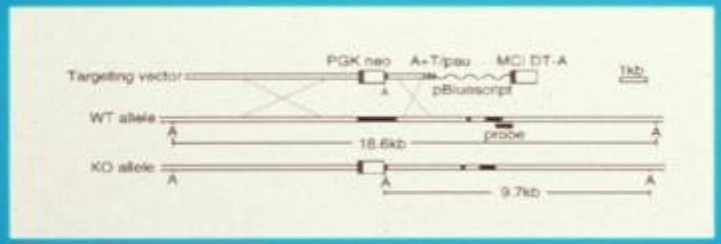
東京大学医学研究科　廣川信隆


a
Distal dendrite
Rough endoplasmic reticulum
Golgi apparatus
Axon hillock
Transporting vesicle
RNA-containing granule
Nucleus
Initial segment
Various transporting vesicles
Growth cone
KIF2A
Microtubules
Axon collateral
Synaptic terminal
Synaptic vesicle
Proximal dendrite
Microtubule
KIF
Axon
Cell body
Multivesicular body-like organelle
Dendritic spine
Dendrites
b
APP
JIPs
APOER2
KLC
Mitochondrion
Fodrin
KAP3
Synaptic vesicle precursor
Microtubule
KIF5
KIF5
KIF5/KIF1Bα
KIF3
KIF1A/KIF1Bβ
c
AMPA receptor
GRIP1
RNA-containing granule
NMDA receptor
LIN7
LIN2
LIN10
Microtubule
KIF5
KIF5
KIF17

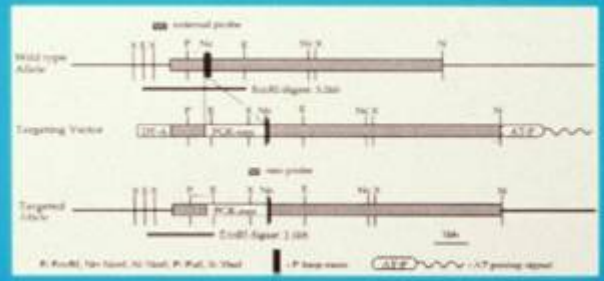
KIF3 Complex
= KIF3A + KIF3B + KAP3



kif3B Knockout



kif3A Knockout

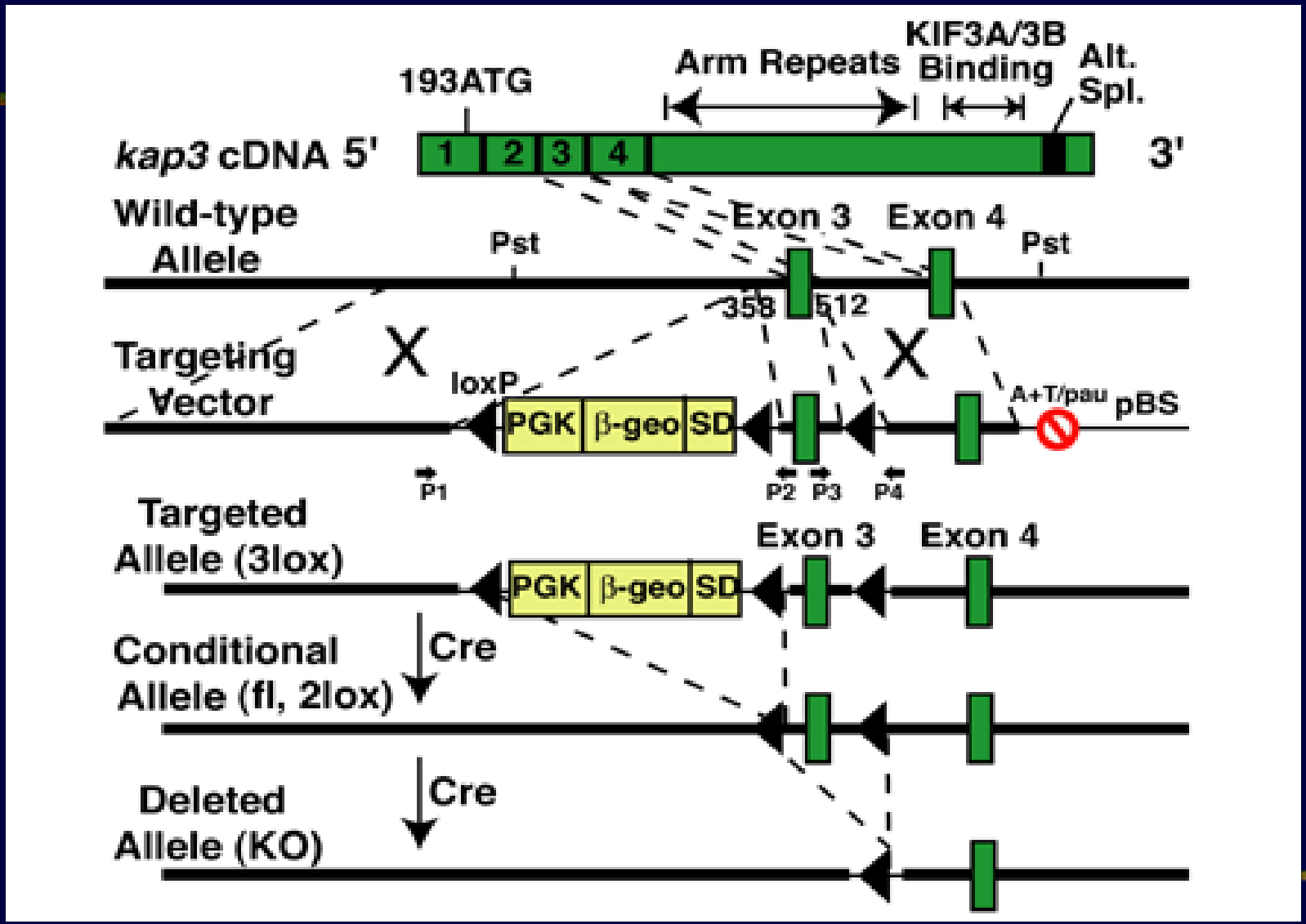


Aizawa et al. **JCB** 1992; Yamazaki et al. **JCB** 1995; Nonaka et al. **Cell** 1998; Takeda et al. **JCB** 1999; Tanaka et al. **Nature** 2005; Okada et al. **Cell** 2005; Hirokawa et al. **Cell** 2006

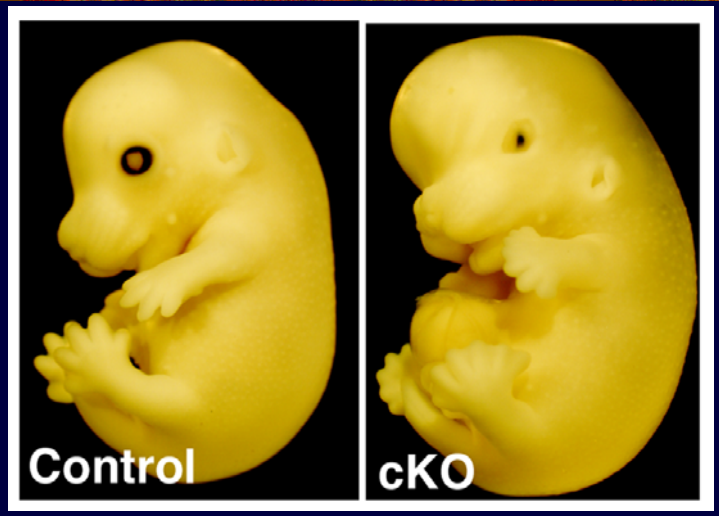
The KIF3 motor transports N-cadherin and organizes the developing neuroepithelium

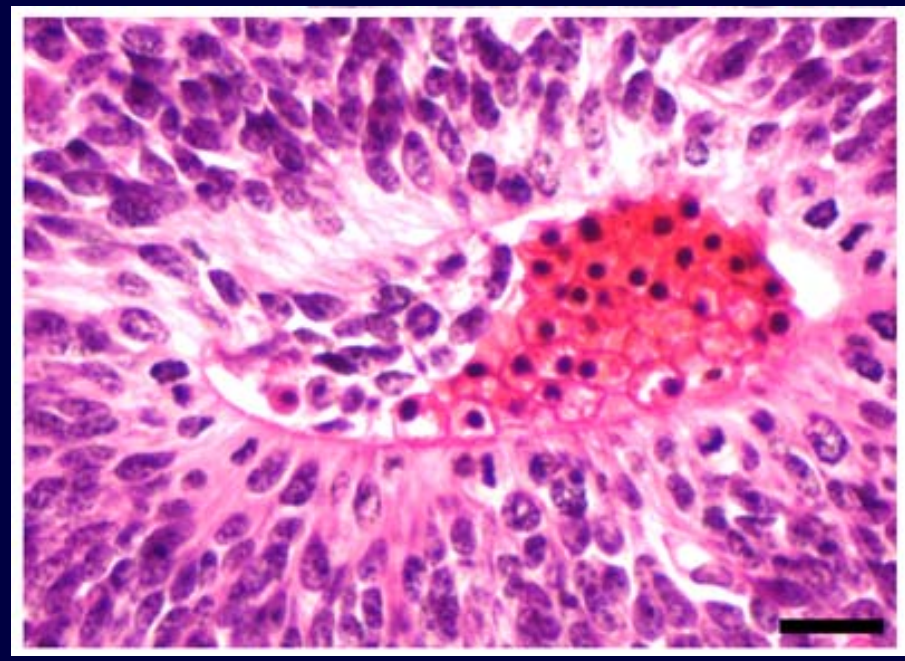
Teng et al. Nature Cell Biol. 7:474-, 2005

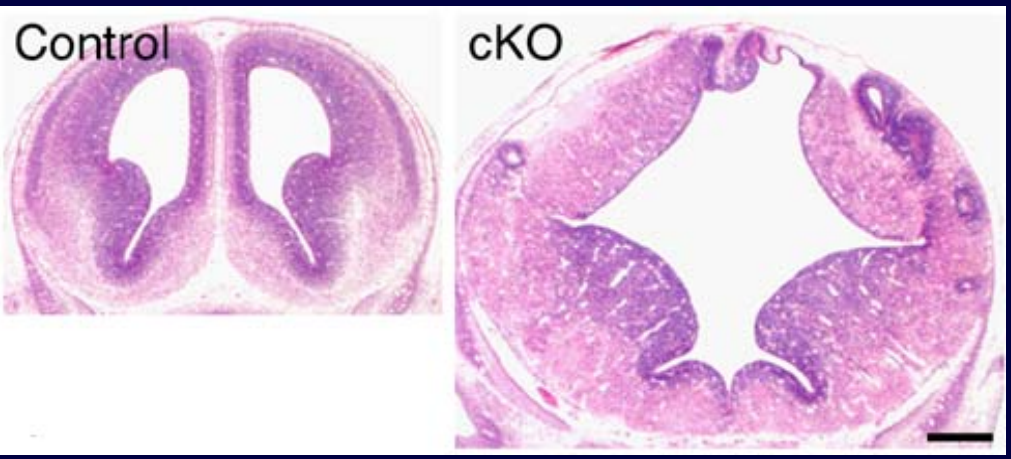
A Schematic view of gene targeting



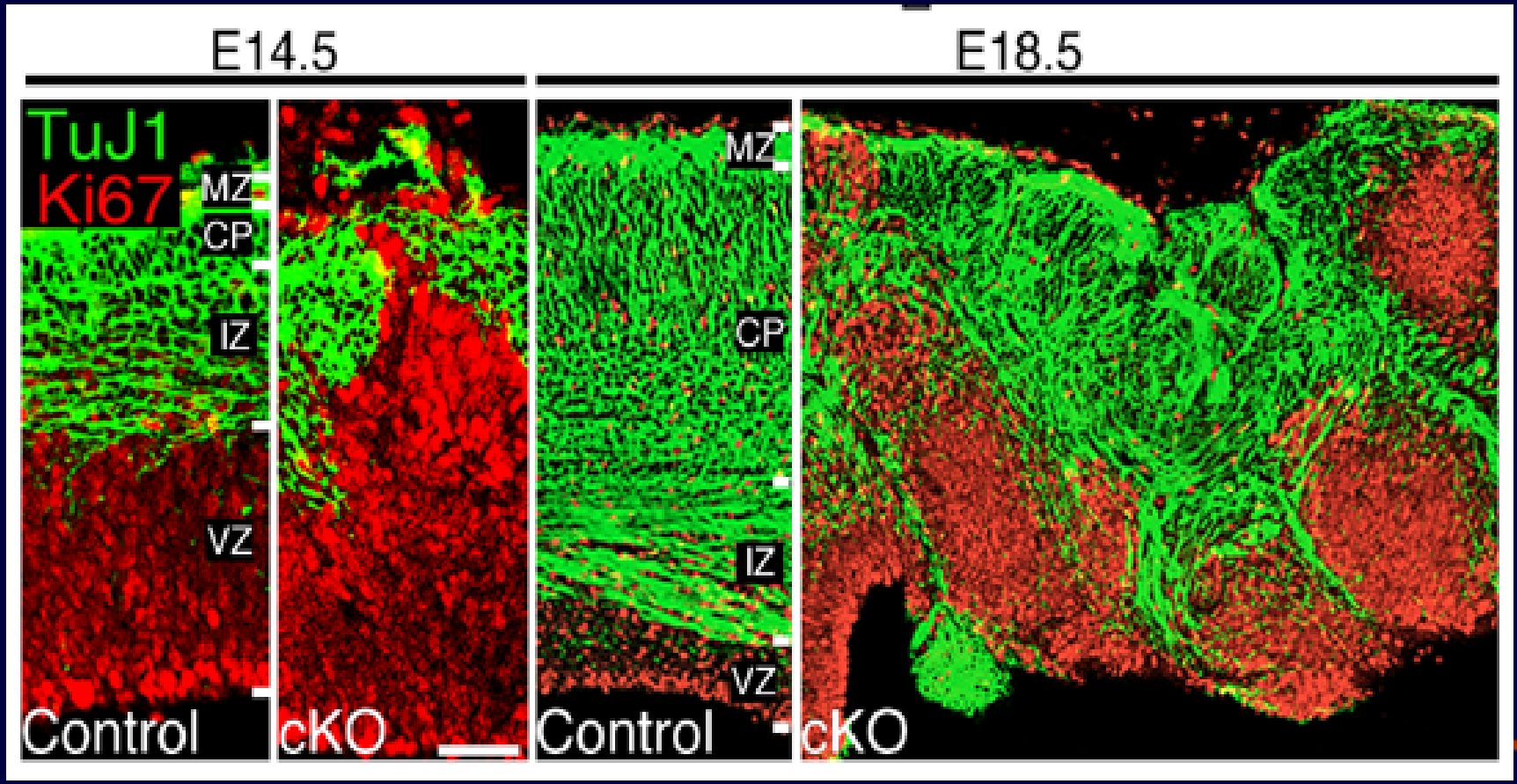
Hypertrophy and invading tumor-like rosette phenotype of kap3 cKO mouse



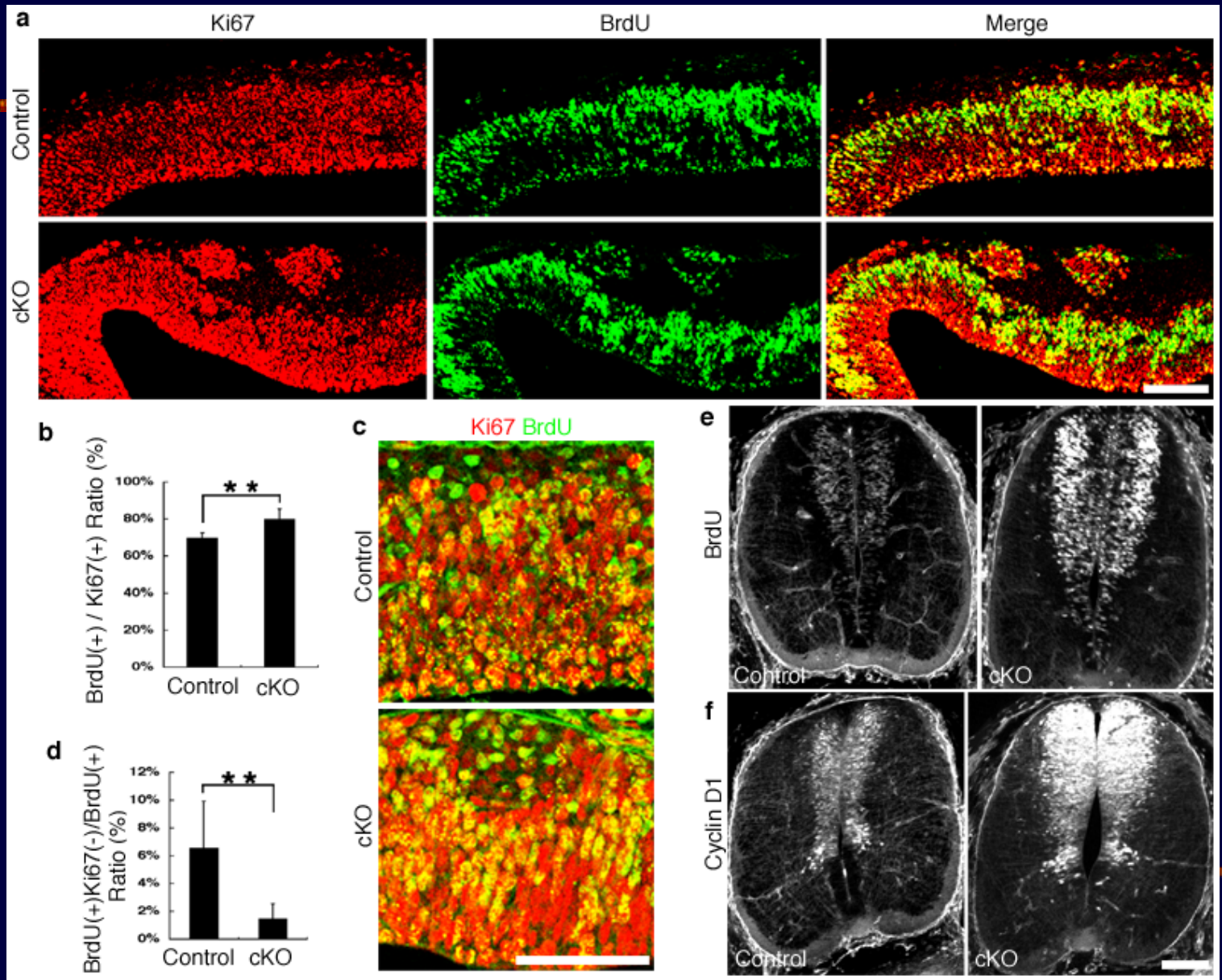




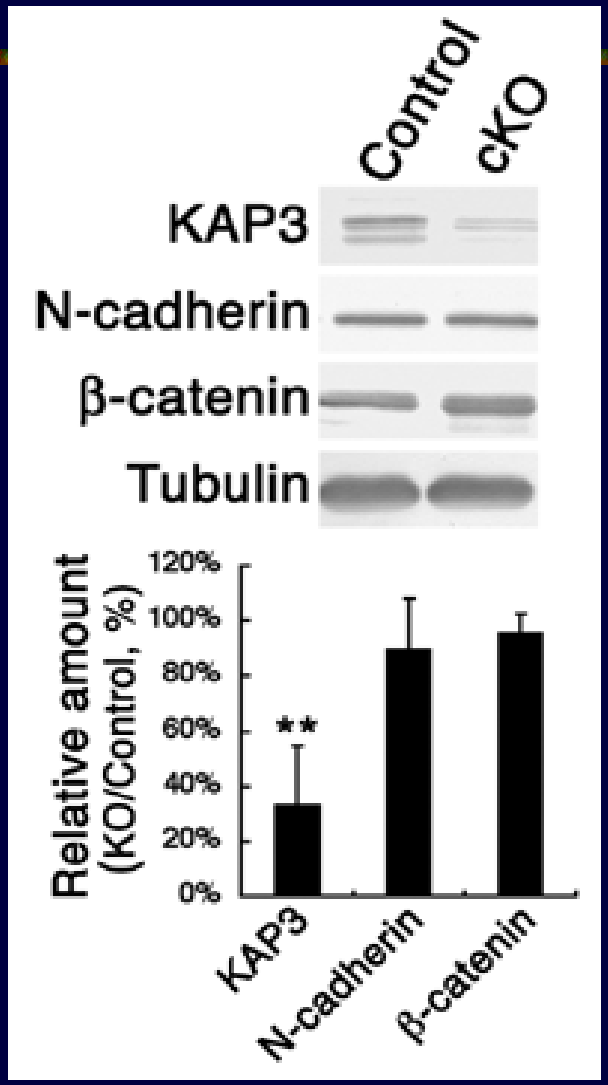
KAP3-deficient leads to malignant transformation of neuroepithelium

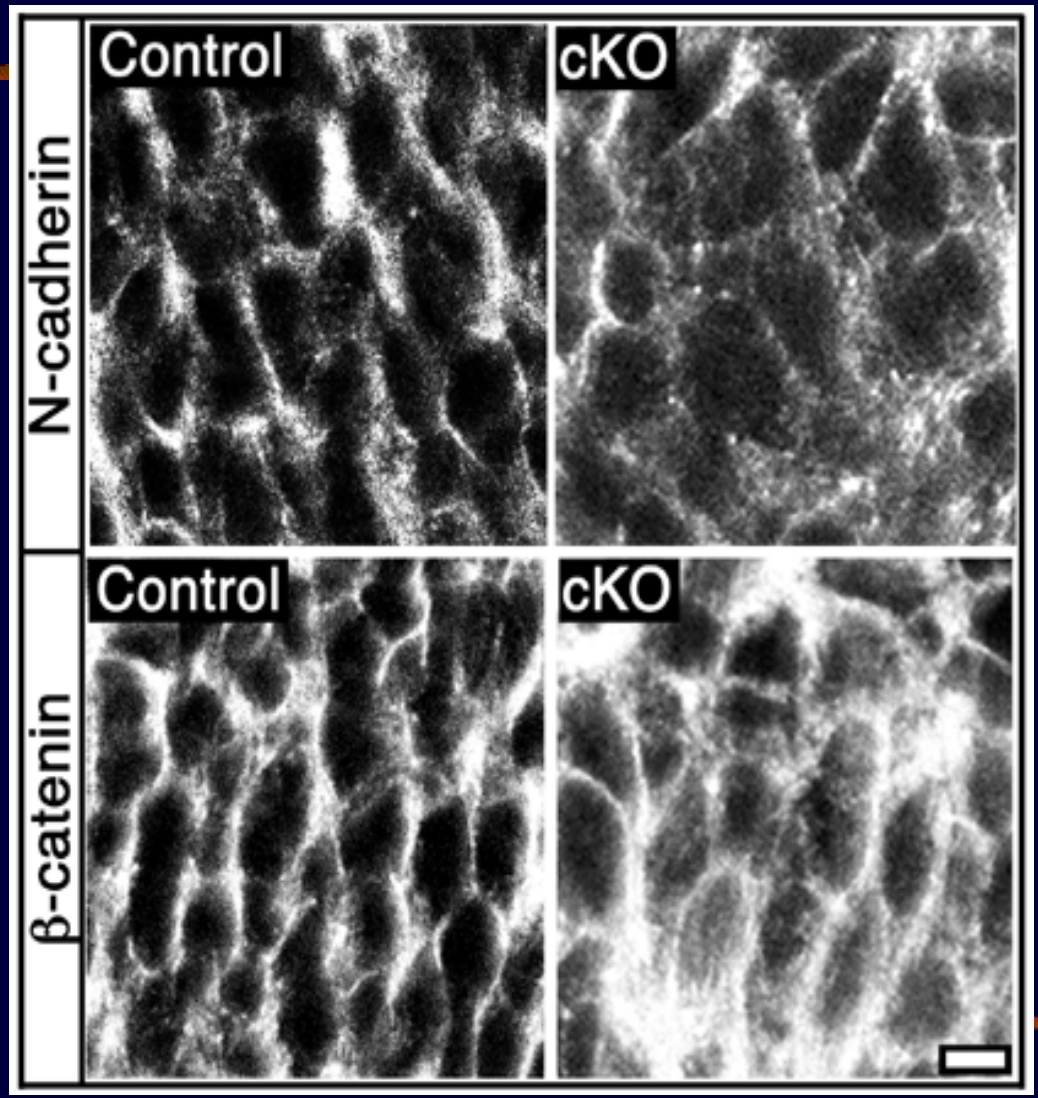


Enlargement of the Neural Progenitor Pool in cKO Mouse Brain

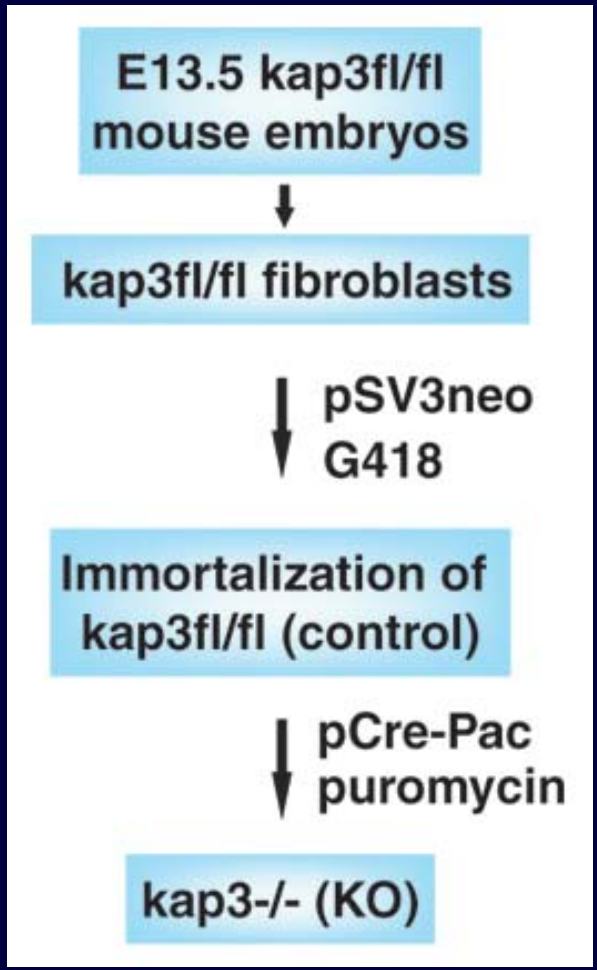


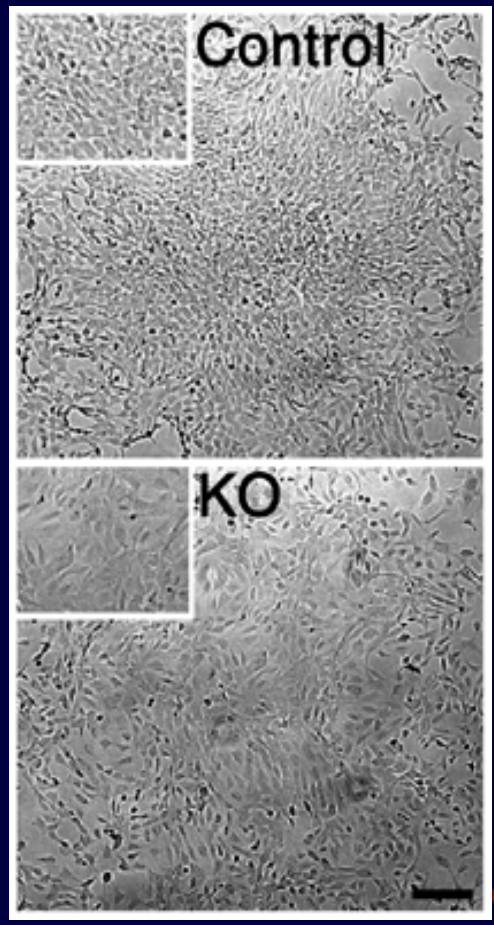
N-cadherin and beta-catenin levels are reduced from cell perphery in cKO brain



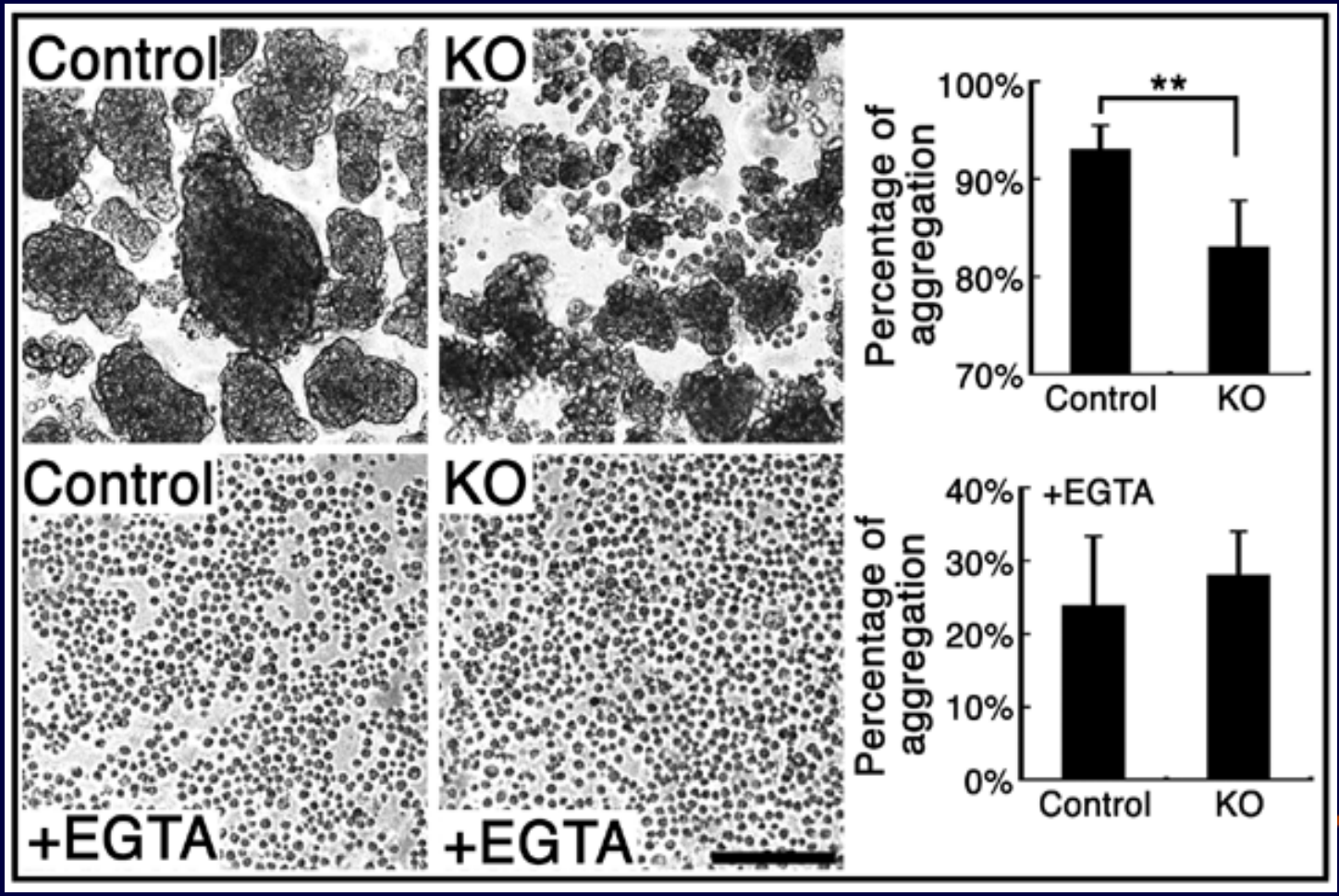


Establishment of kap3fl/fl and kap3-/- embryonic fibroblast cell lines

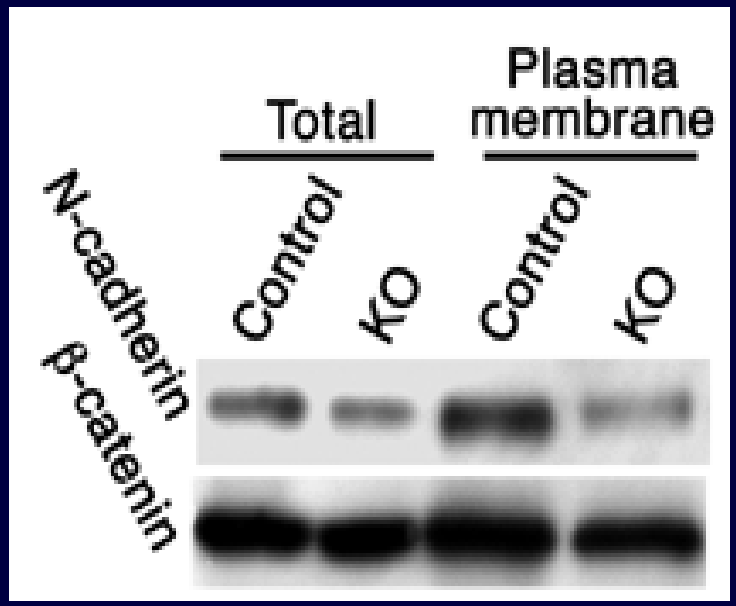


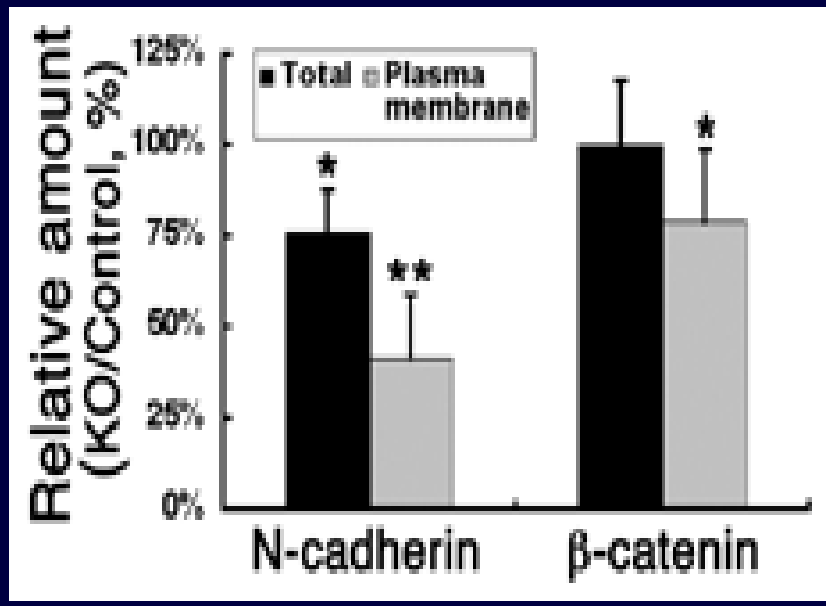


Impaired Ca^{2+}-dependent cell adhesion in kap3-/- MEFs

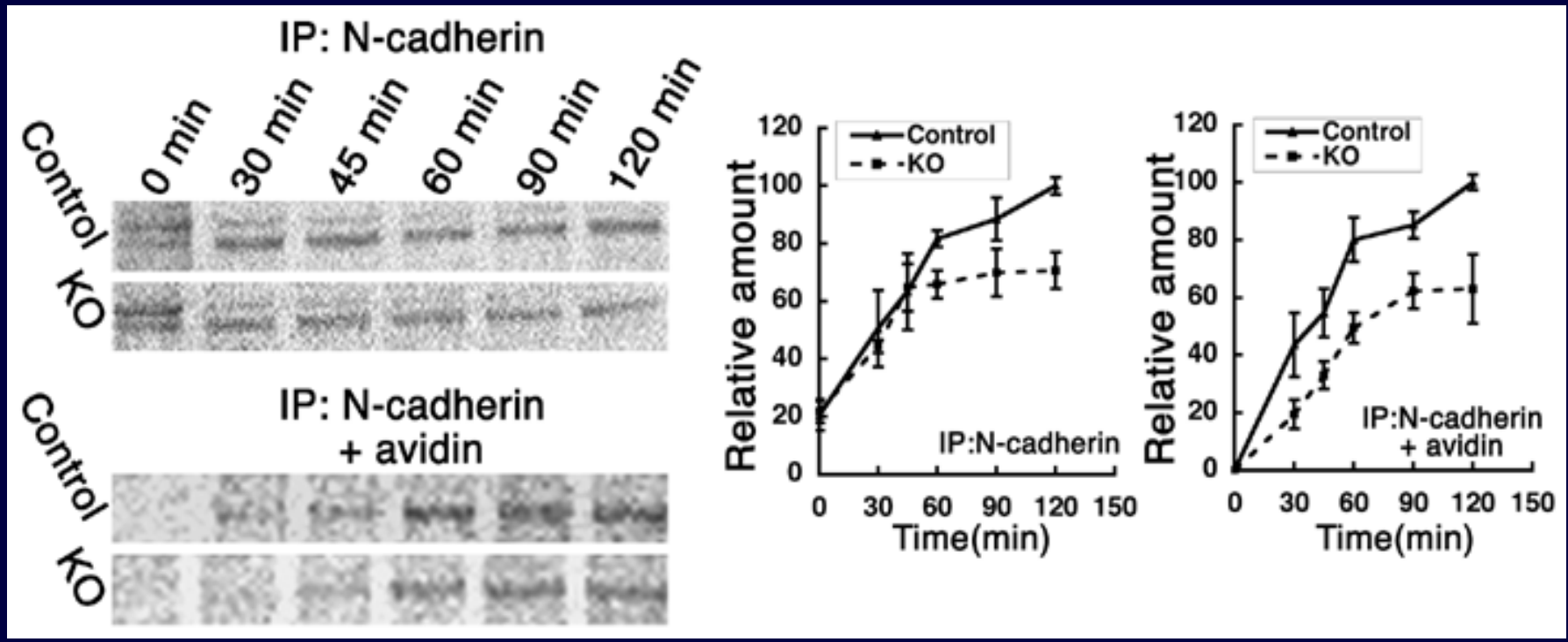


Reduced N-cadherin and Beta-catenin levels from cell periphery

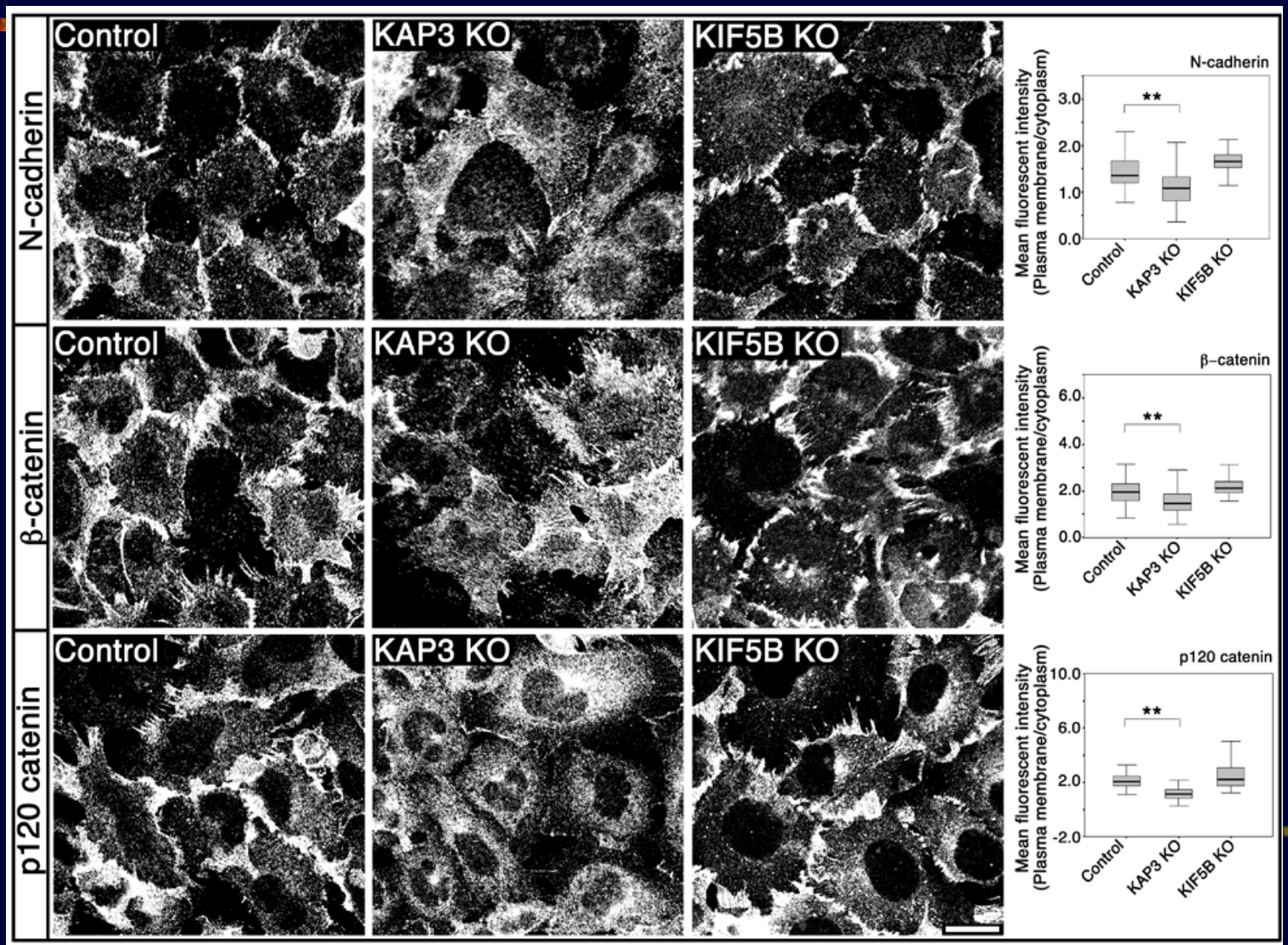




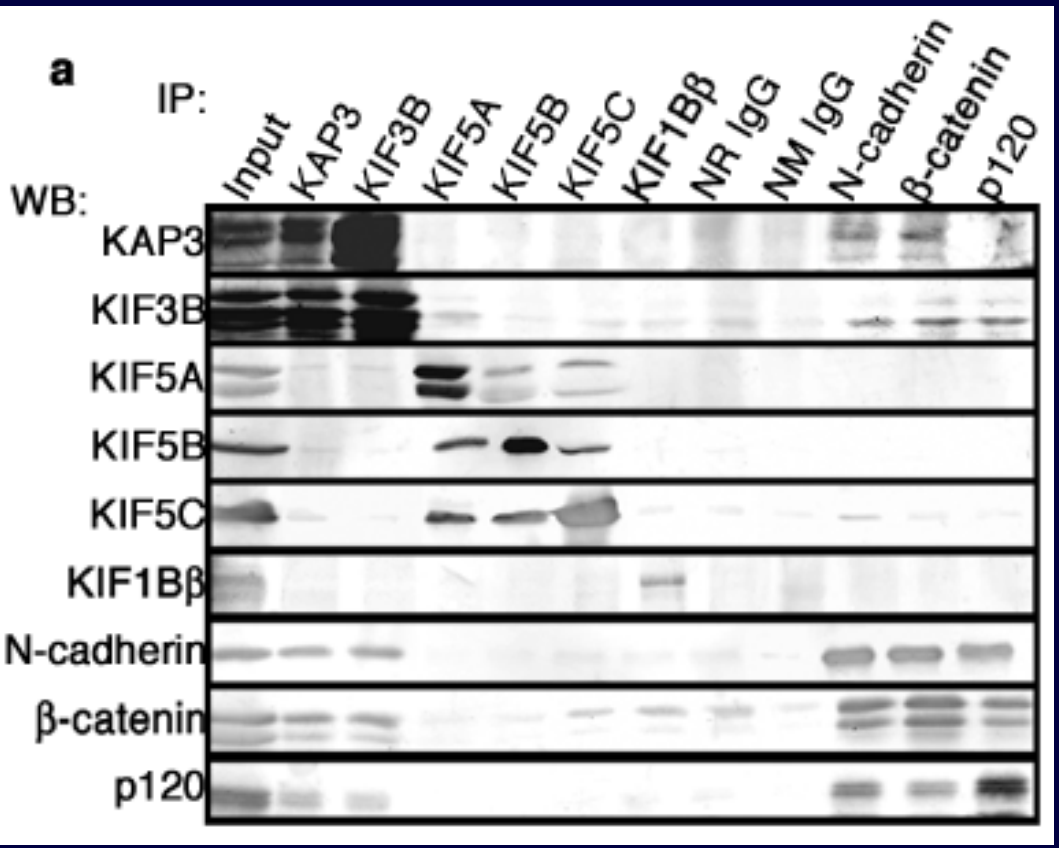
Diminished arrival of newly synthesized N-cadherin to the plasma membrane

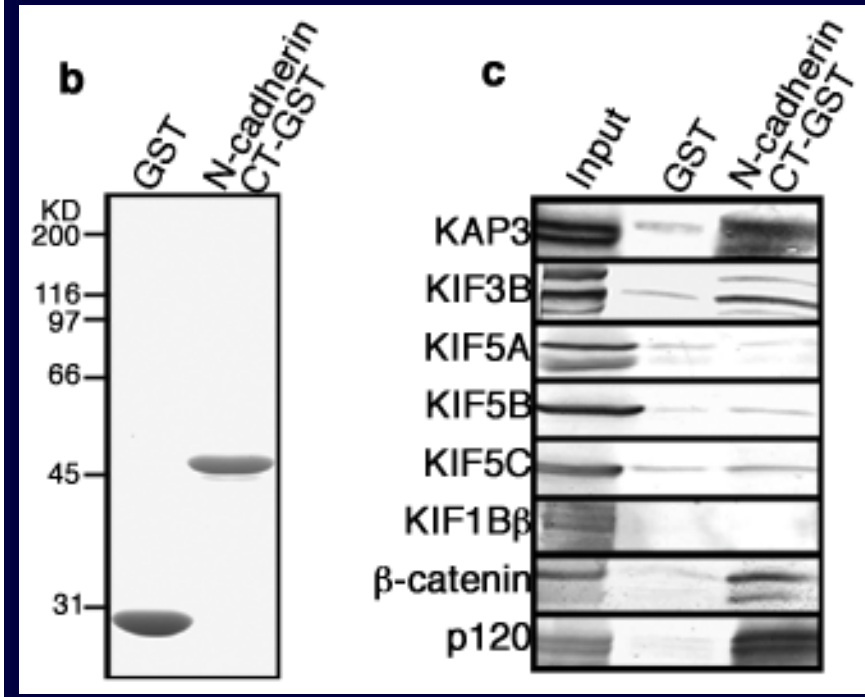


Impaired plasma membrane targeting of N-cadheri, Beta-catenin , and p120 in KAP3-deficient Cells

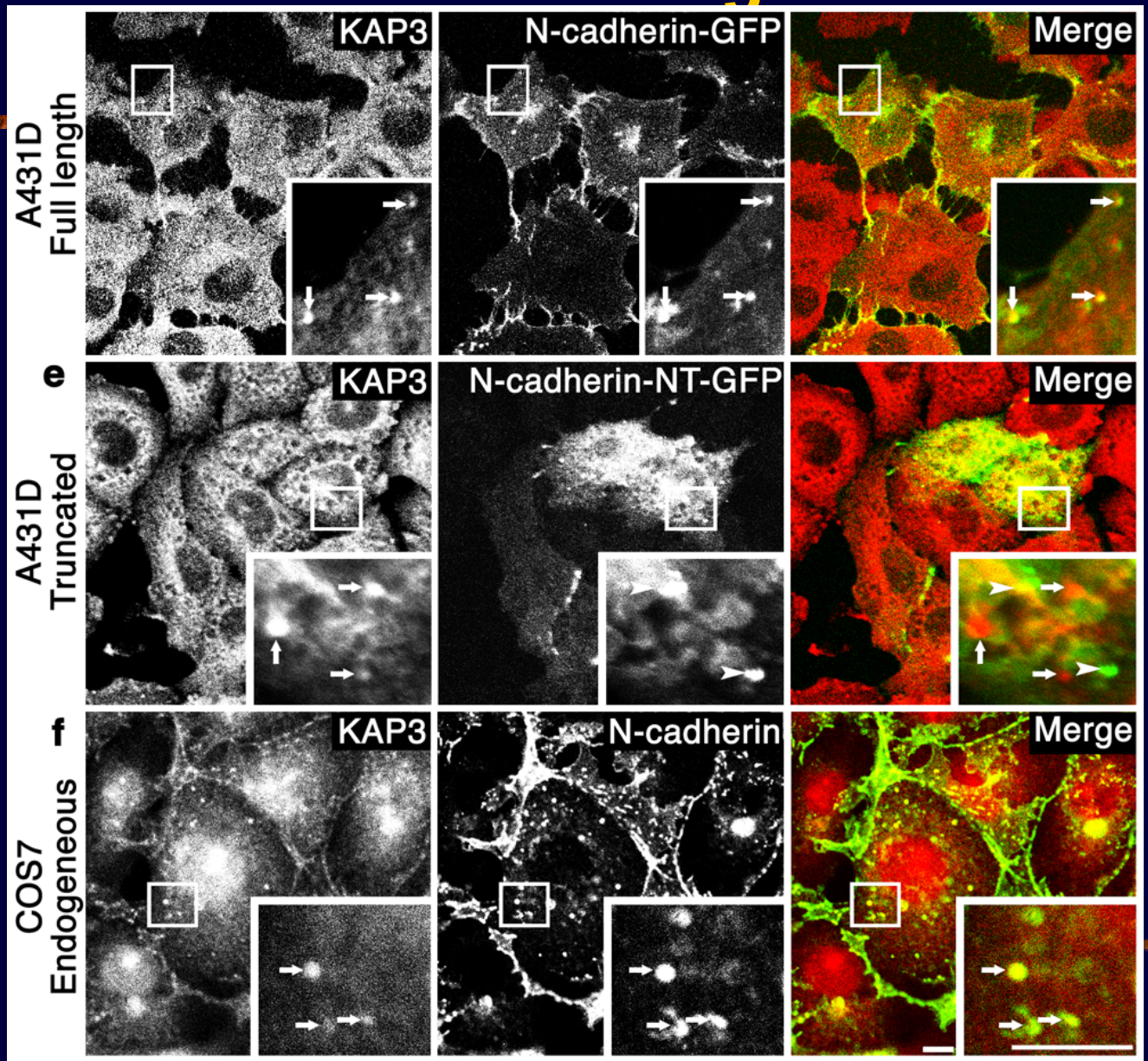


KIF3A/3B/KAP3 heterotrimer associated with N-cadherin complex

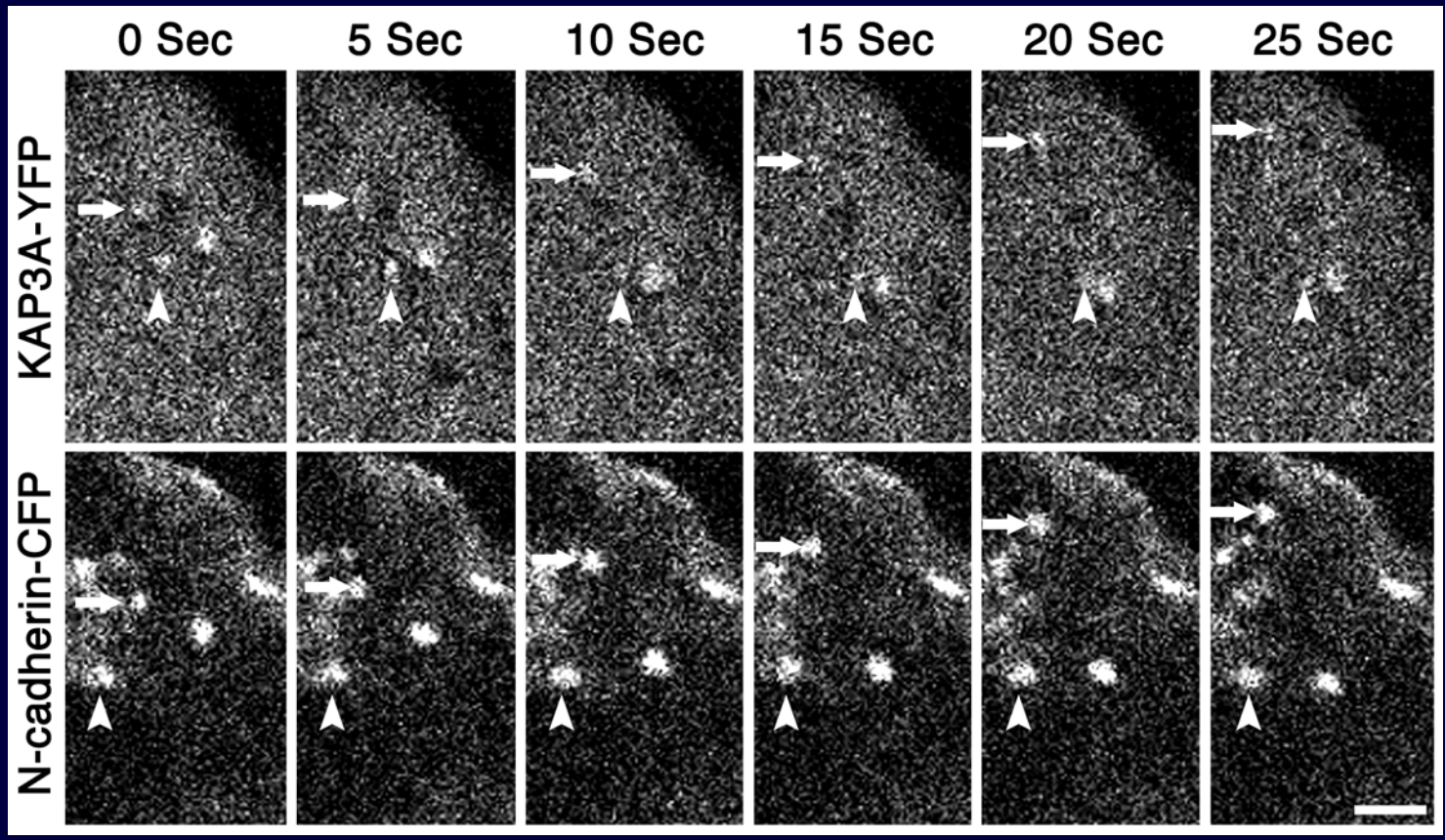




Colocalization of KIF3/KAP3 with N-cadherin containing vesicles



KAP3 and N-cadherin moving together in A431D cells



KAP3 and N-cadherin moving together in A431D cells

QuickTimeý Ç²
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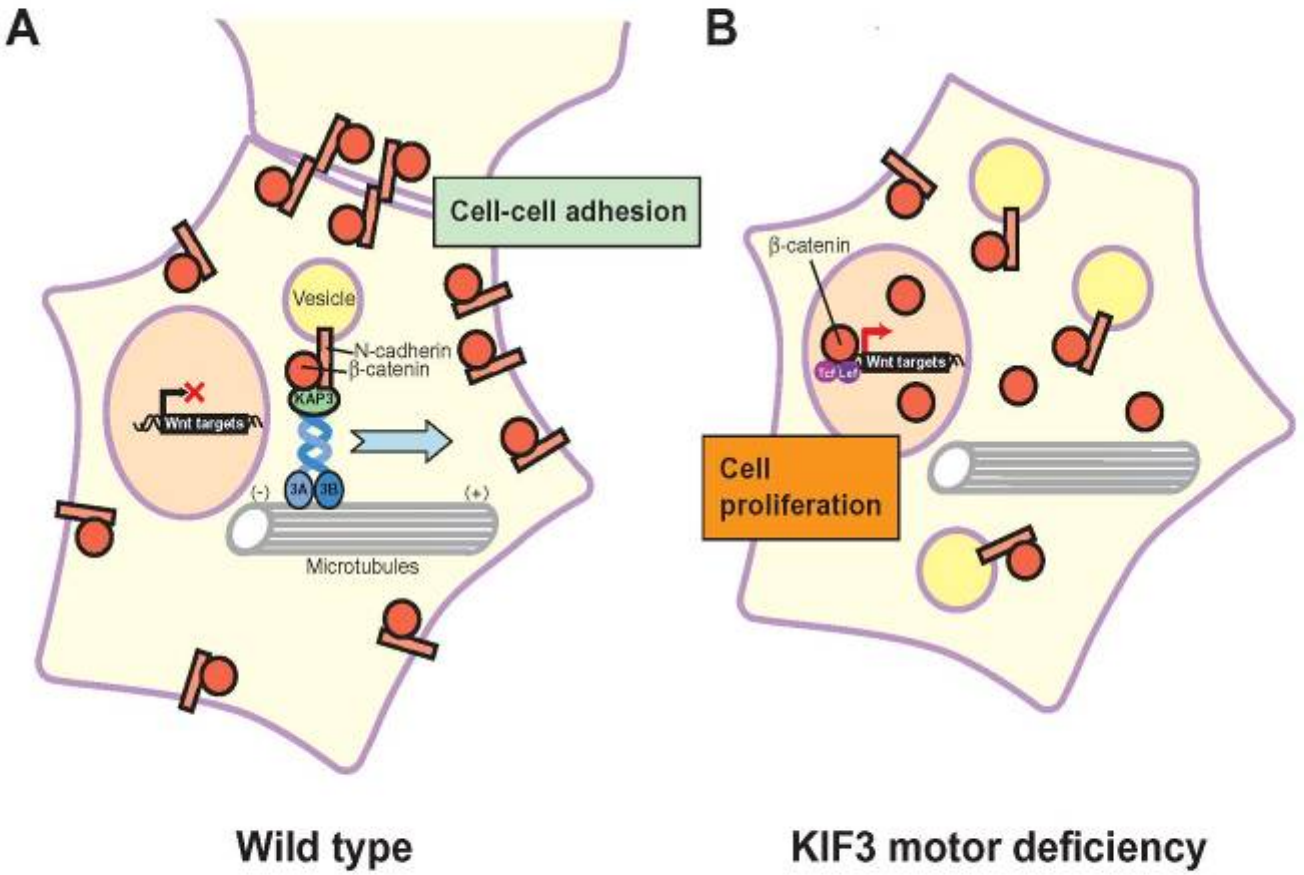
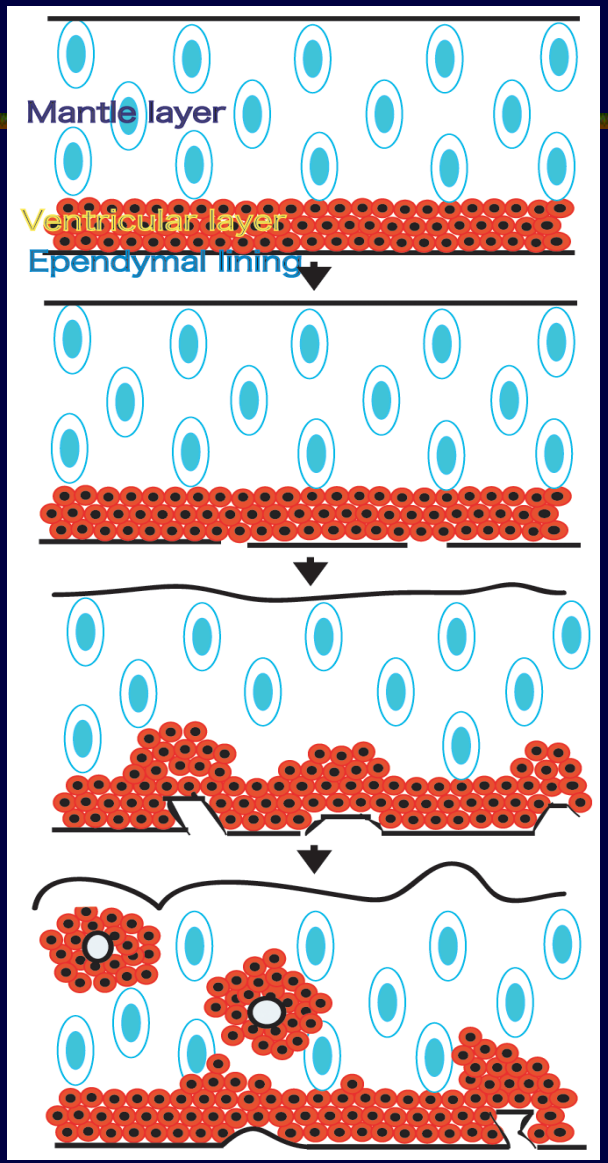
Ç™Ç±ÇÃÉsÉNÉ`ÉÉÇ¾å©ÇÈÇ…ÇÕïKóvÇ-ÇÍÅB


A
B
Cell-cell adhesion
Vesicle
N-cadherin
β-catenin
KAP3
Wnt targets
3A
3B
(-)
(+)
Microtubules
β-catenin
Tcf Lef
Wnt targets
Cell proliferation
Wild type
KIF3 motor deficiency

Mechanism of phenotype of cKO brain formation



KIF3 motor-deficient

↓

Impaired post-Golgi transport of N-cadherin

↓

Loss balance between proliferation and cell-cell adhesion

↓

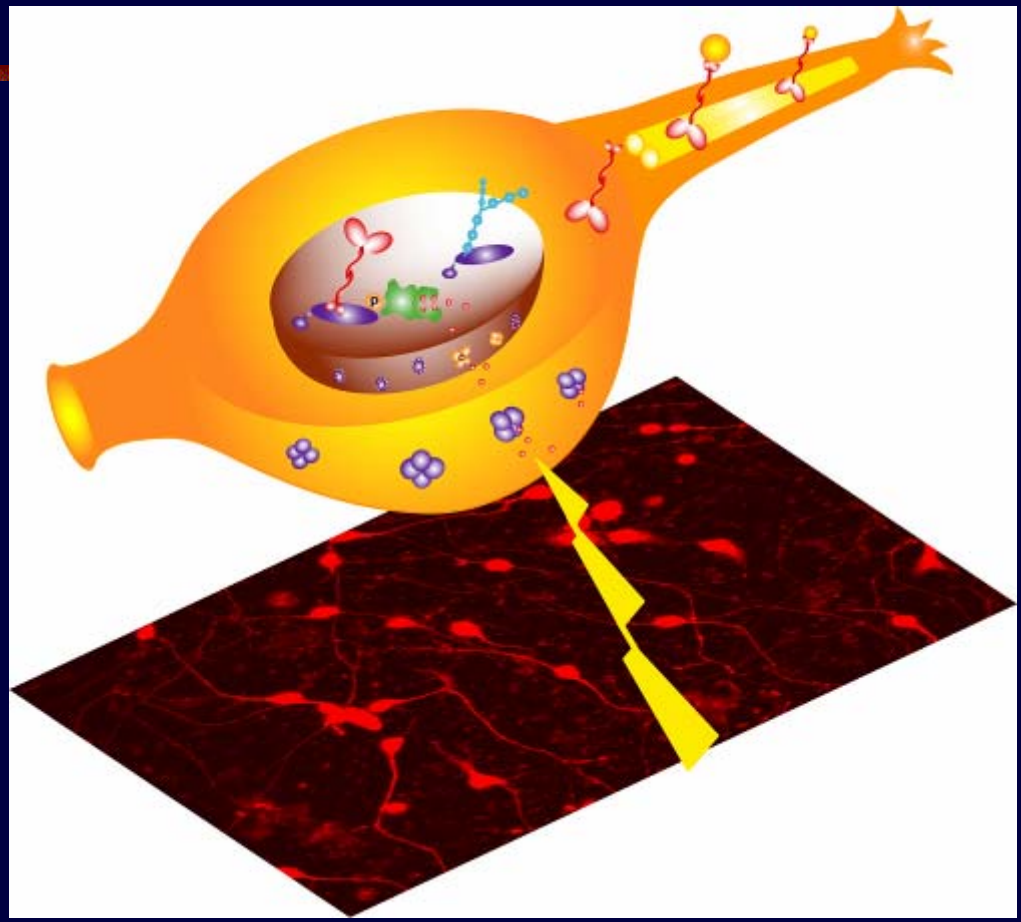
Expand progenitor pool
Decreased adhesion of neuroepithelium

↓

Rosette

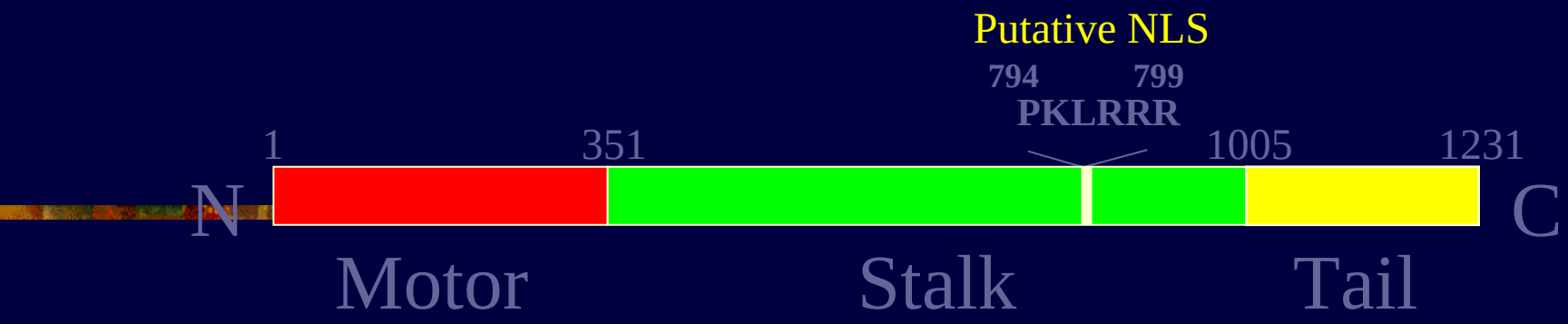
KIF 4 and Activity Dependent Neuronal Survival

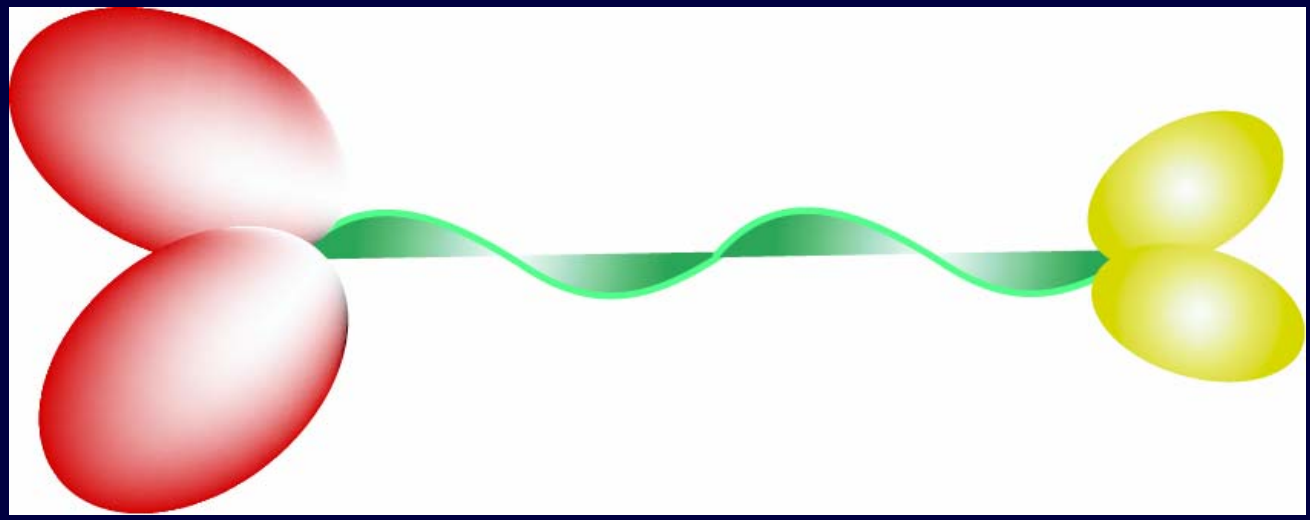
KIF4 regulates activity-dependent neuronal survival by suppressing PARP-1 enzymatic activity



R. Midorikawa et al.
Cell 125 :371-383, 2006

Structural view of KIF4

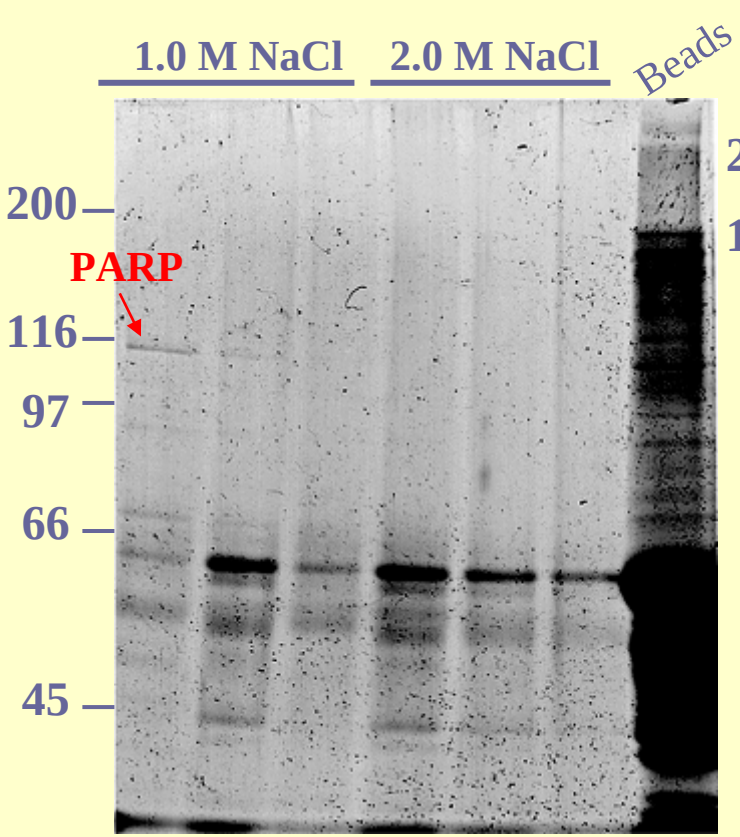




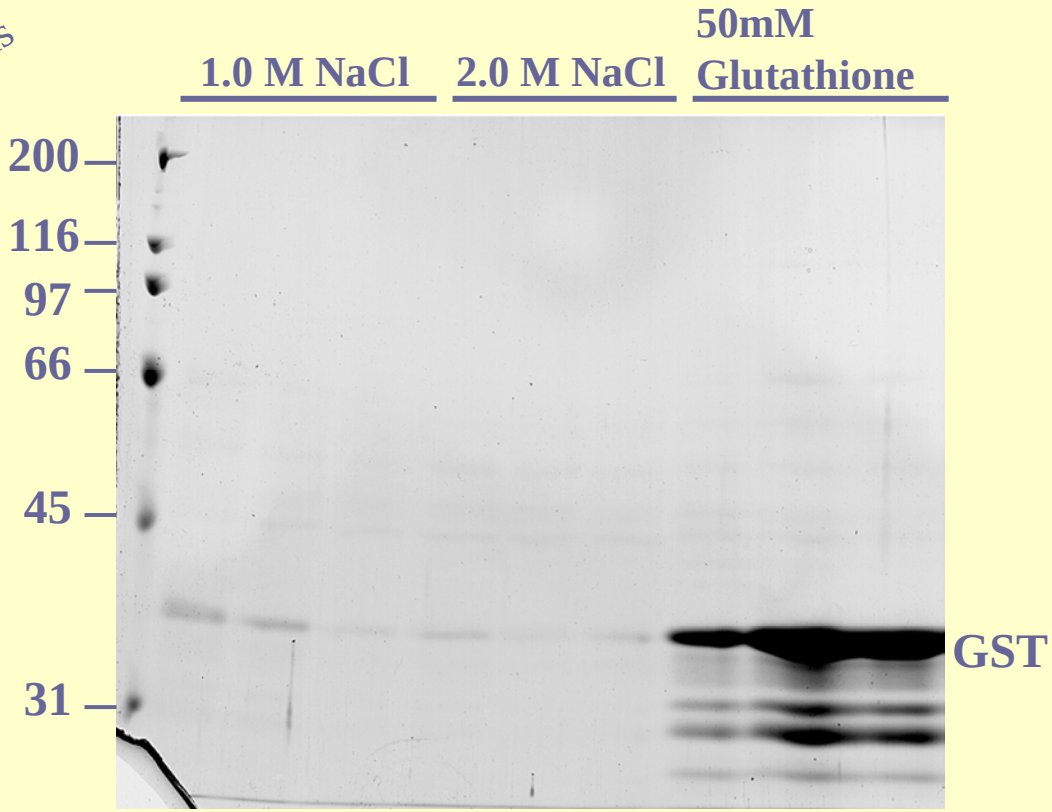
- Tissue distribution : ubiquitous (in juvenile stage)
- Intracellular localization : nuclei and cytosol

Screening of KIF4-binding protein using GST-pull down

(A) Pull Down using GST-KIF4 tail

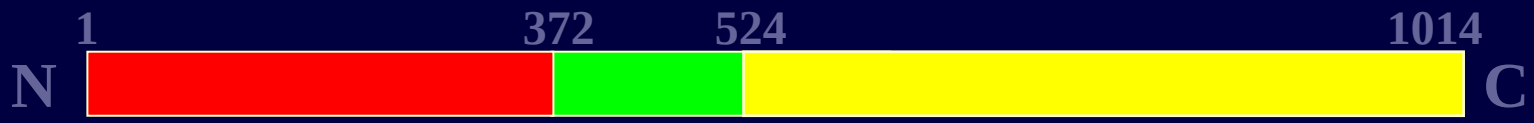


(B) Pull down using only GST



PARP : Poly (ADP-ribose) polymerase

Structural view of PARP

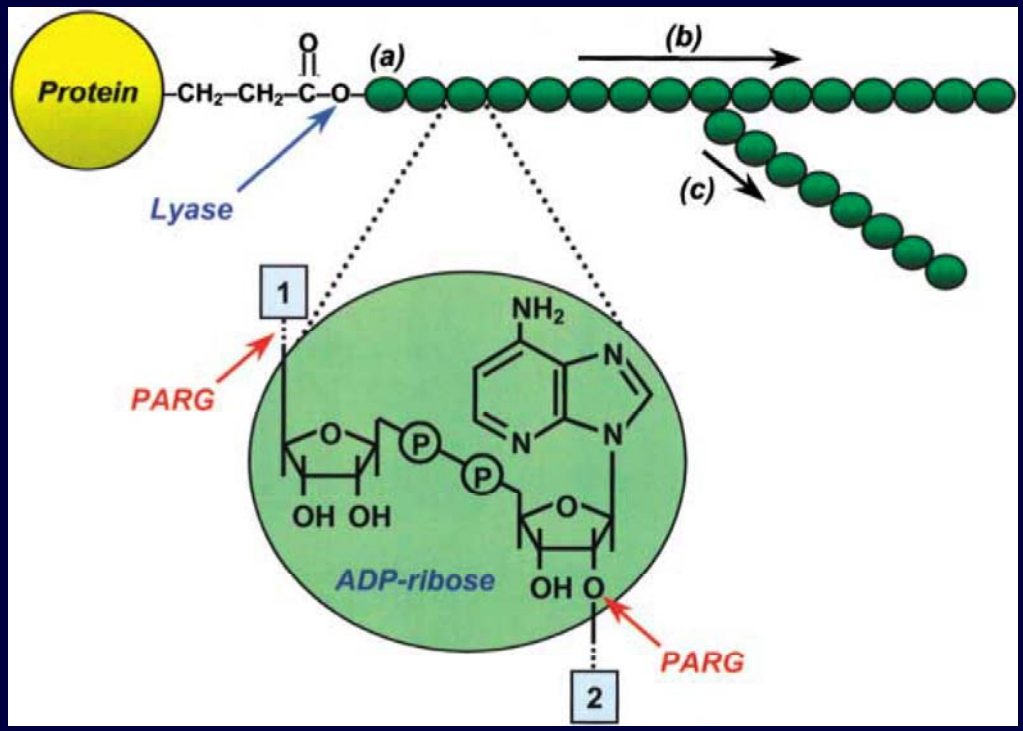


DNA binding domain

Auto-modification domain

Catalytic domain

Poly ADP-ribosylation for acceptor protein by PARP

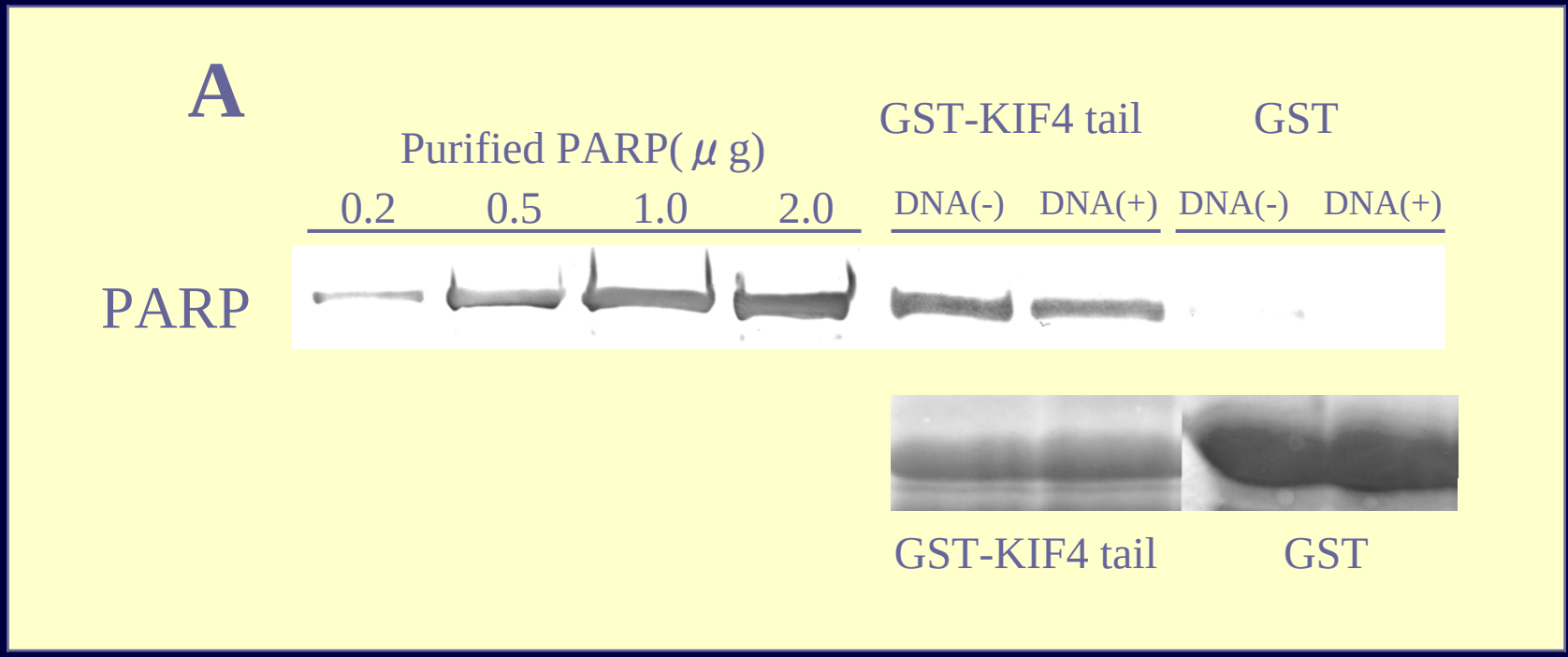


Modifying

- activity of various transcription factors
- chromatin structure

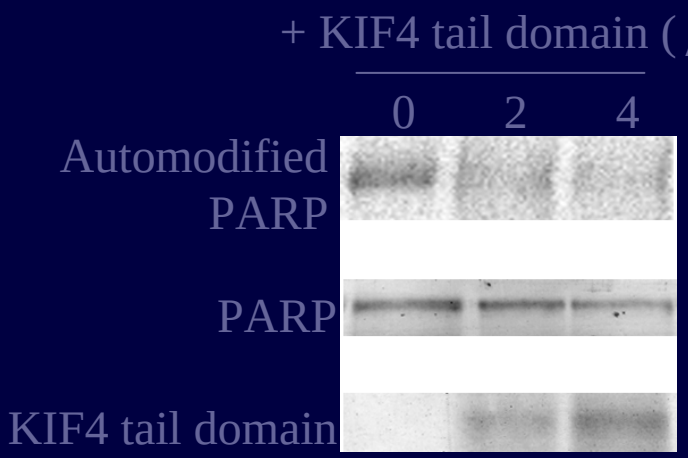
In-vitro binding assay

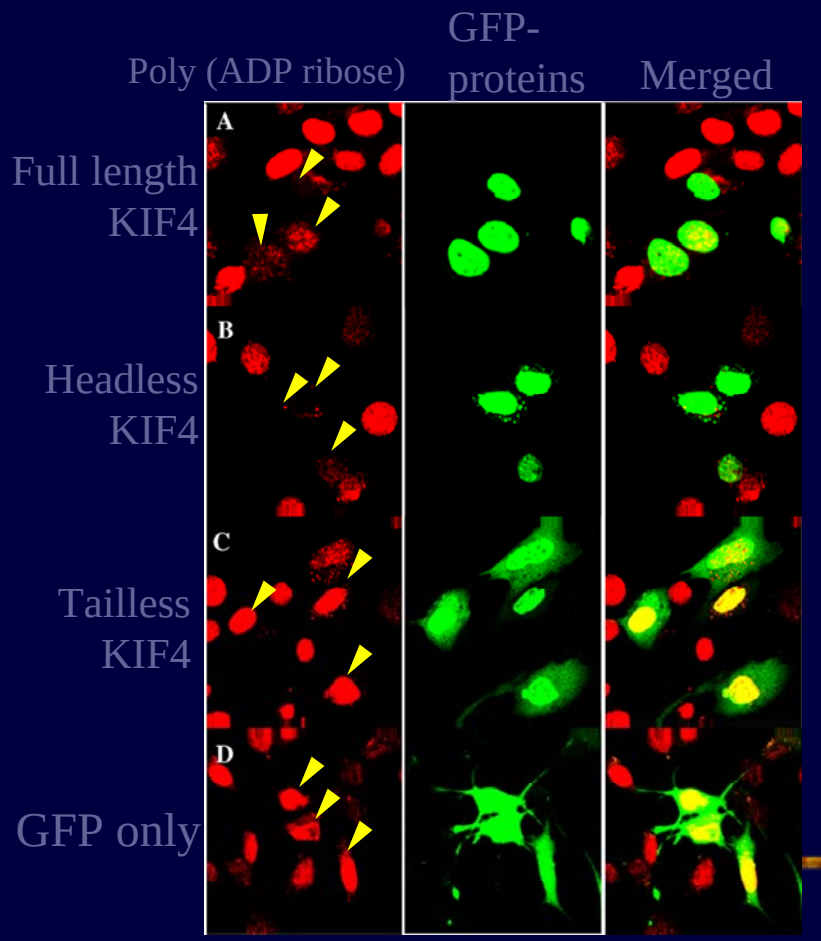
Binding between tissue-purified PARP and GST-KIF4 tail



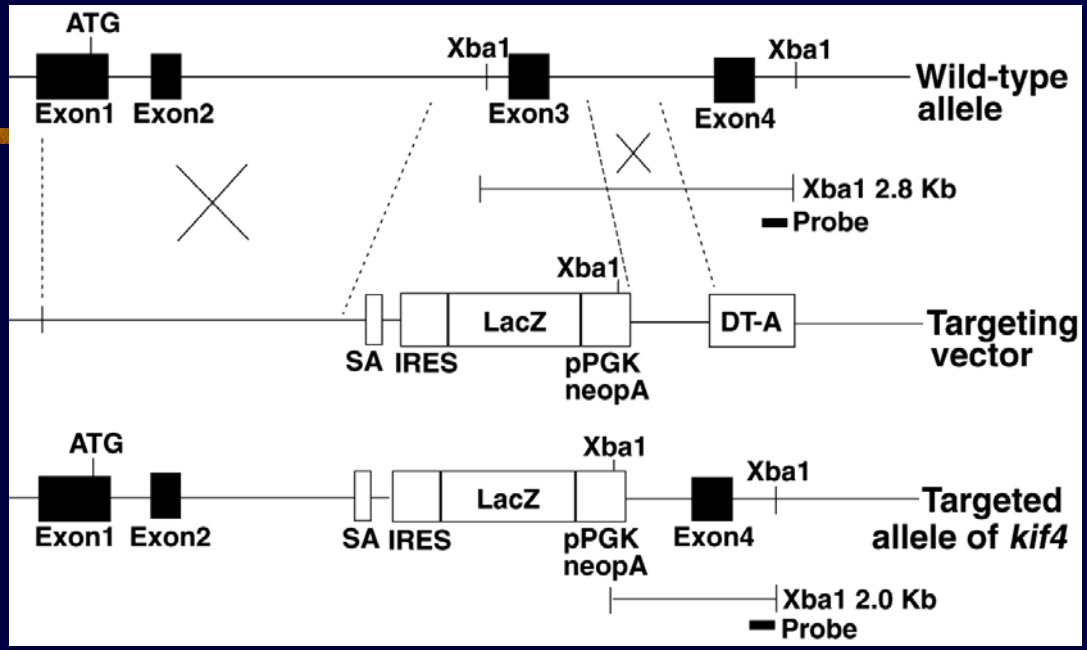
Overexpression of KIF4 deletion mutants in NIH3T3 cells

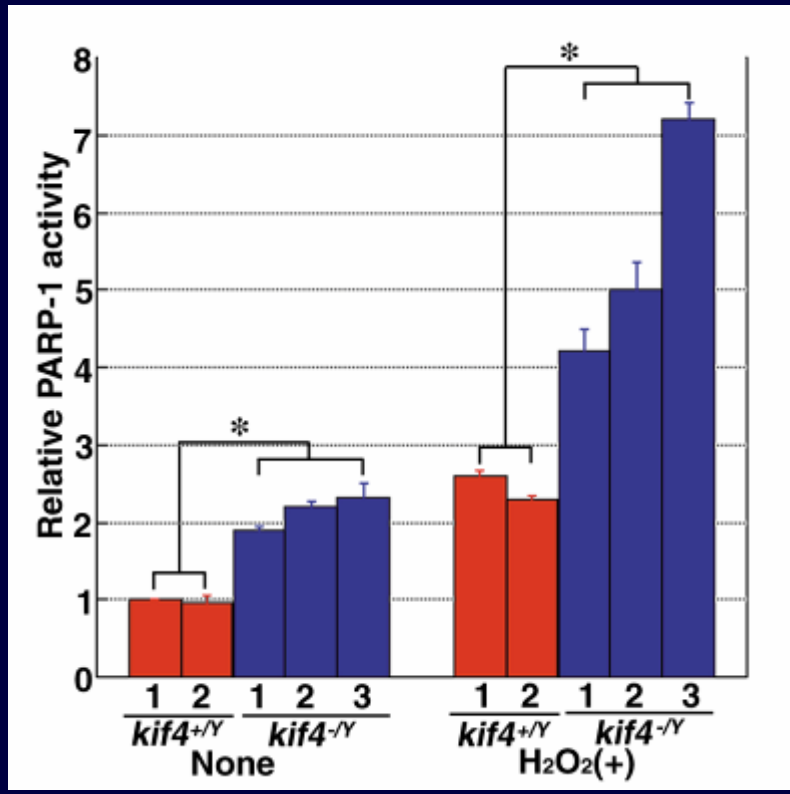
Effect of KIF4 tail on PARP automodification

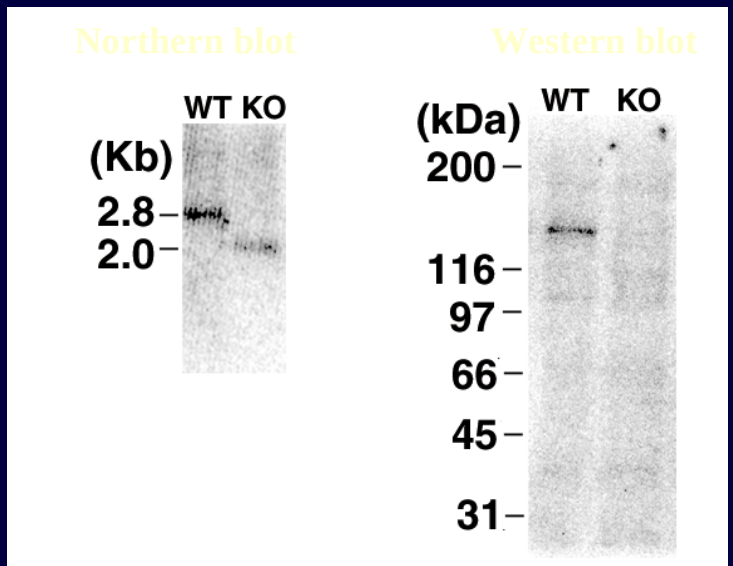




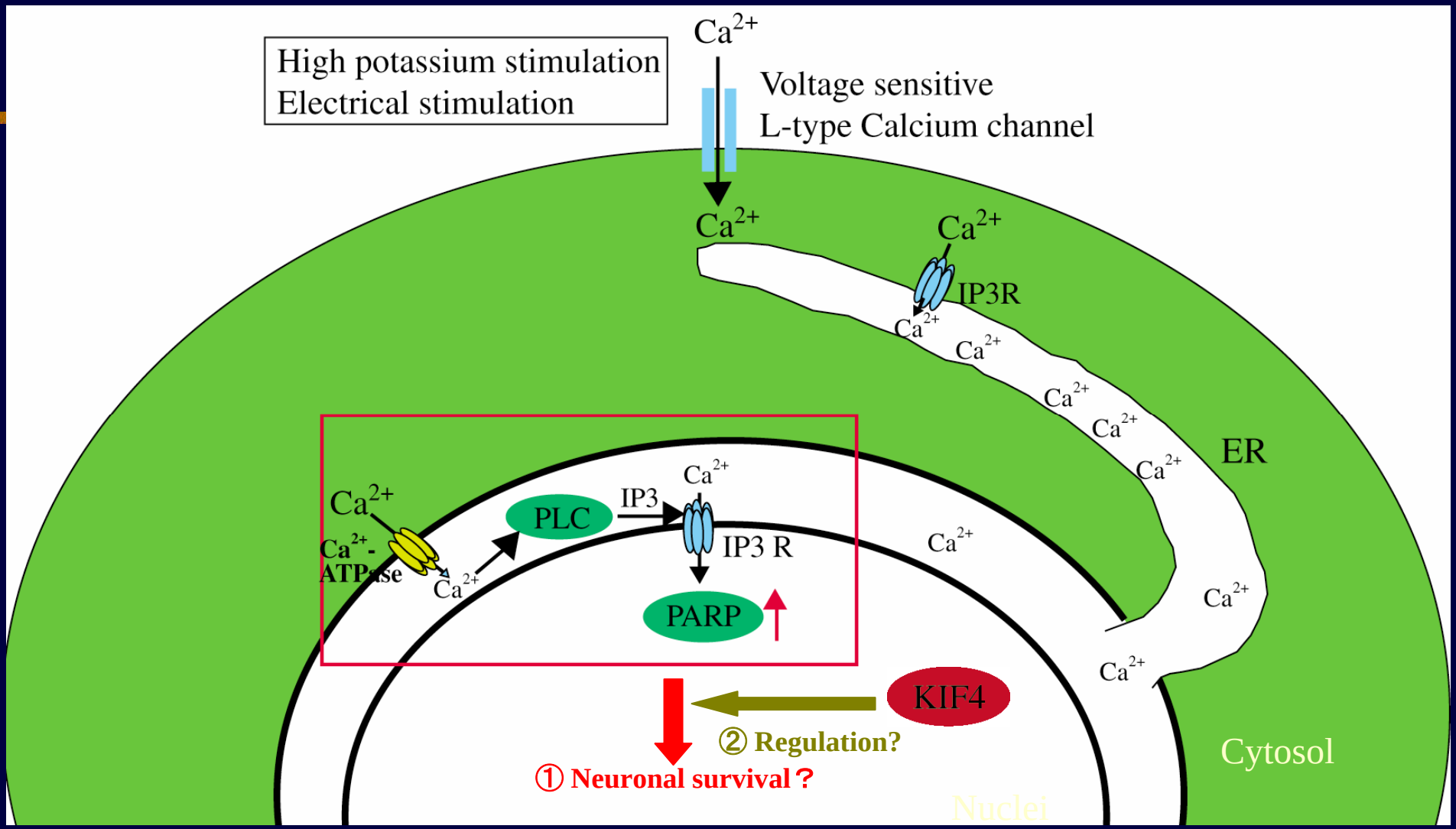
PARP activity of wild-type and kif4 KO ES cells



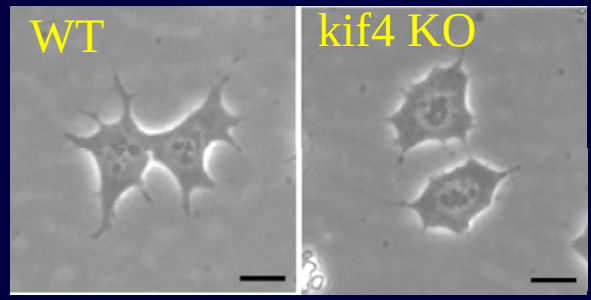




A fast signal-induced activation of poly (ADP-ribose) polymerase: a novel downstream target of phospholipase C.
Homburg et al. J. Cell Biol. (2000)150, 293-307.

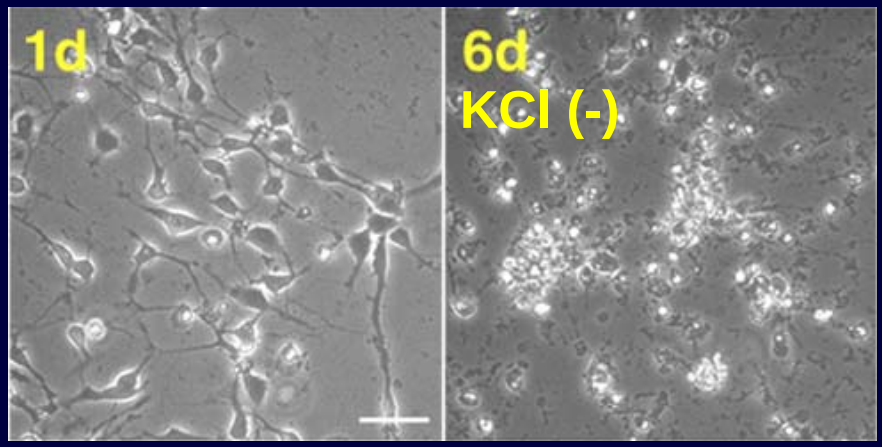


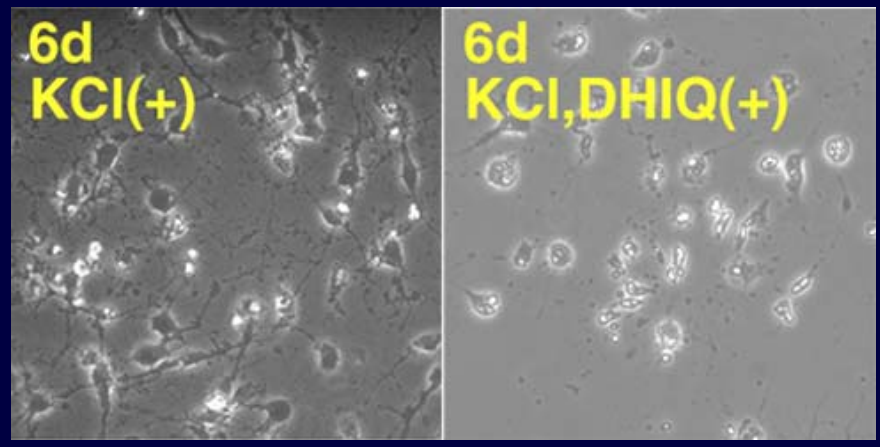
ES cells



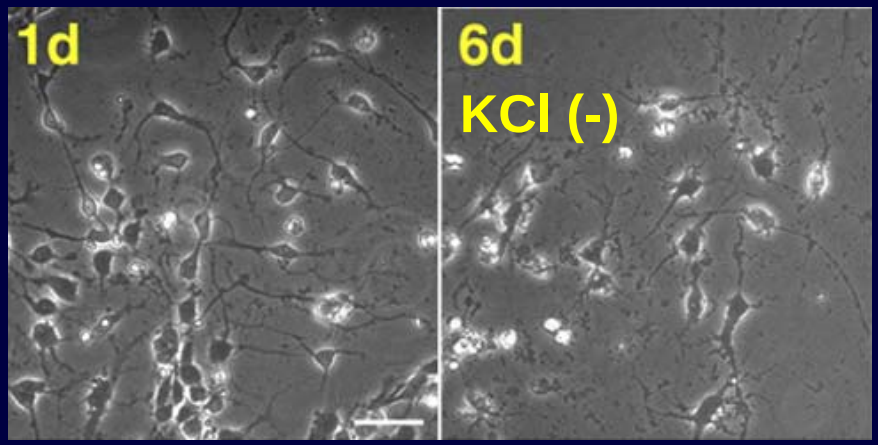
In vitro differentiation into excitatory neurons

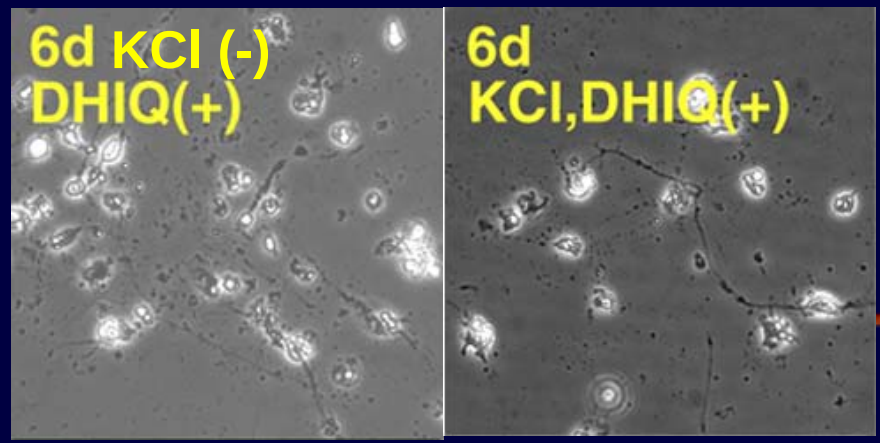
WT neurons



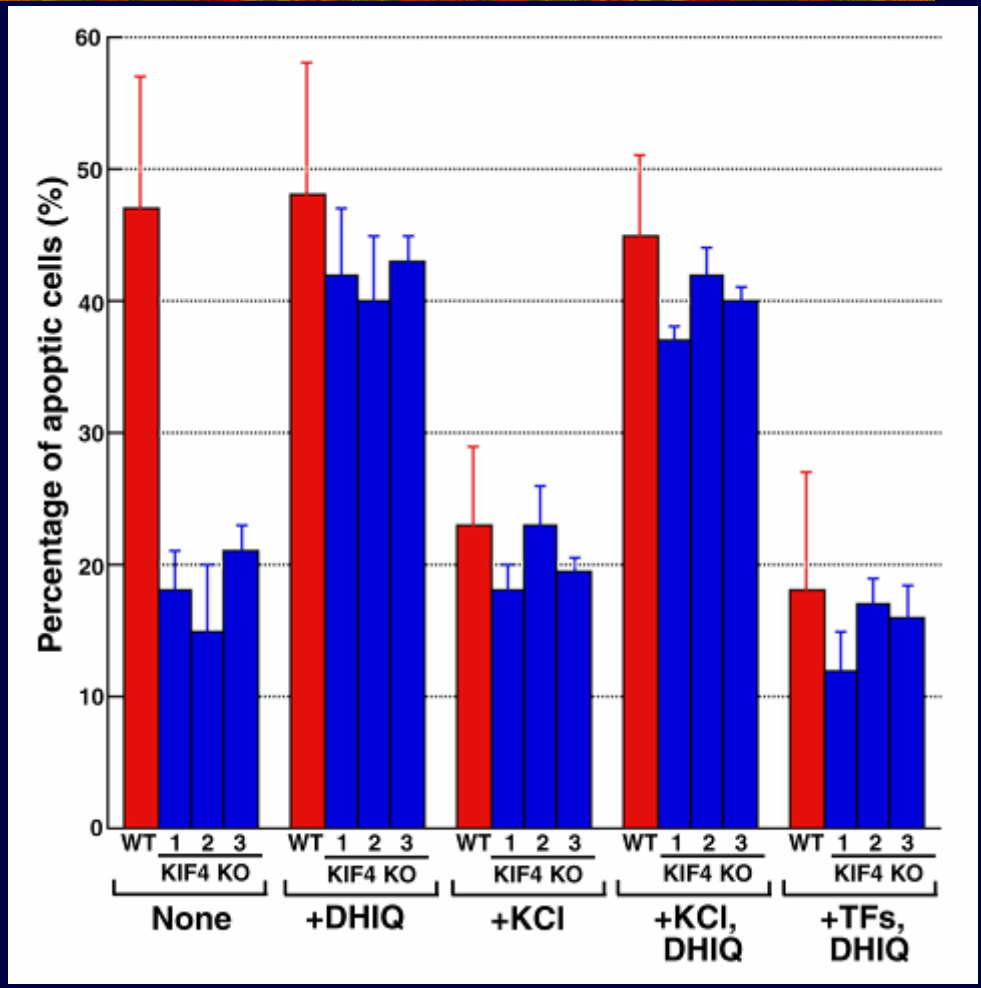


kif4 KO neurons

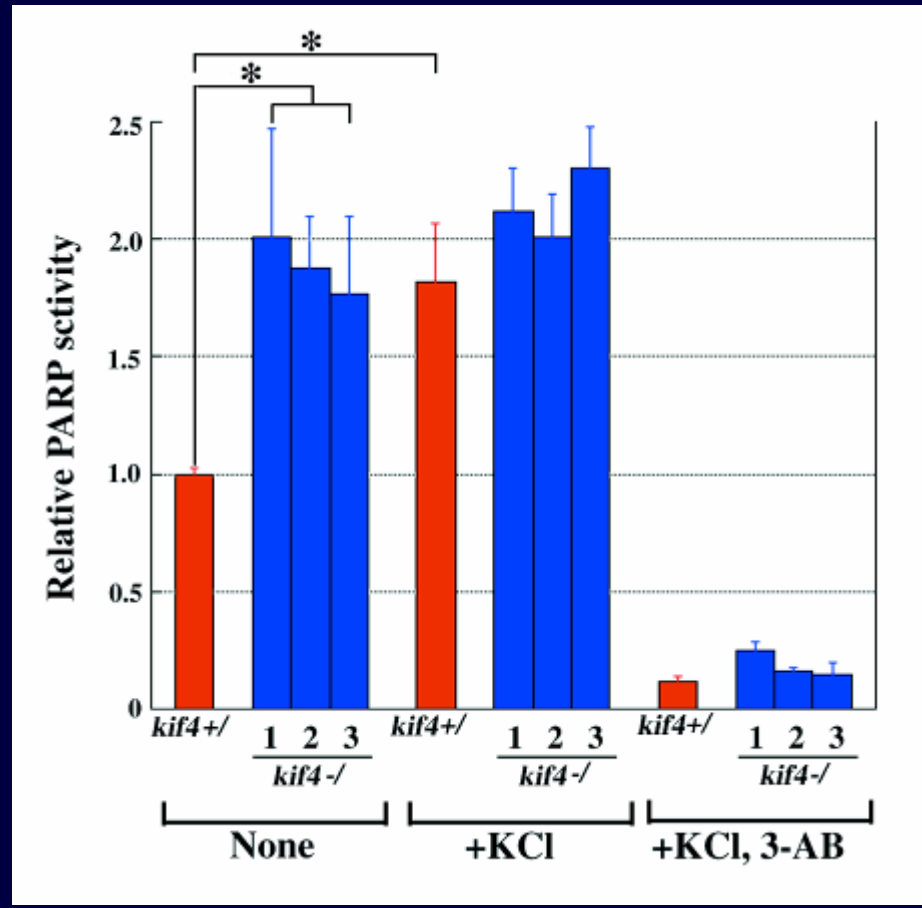




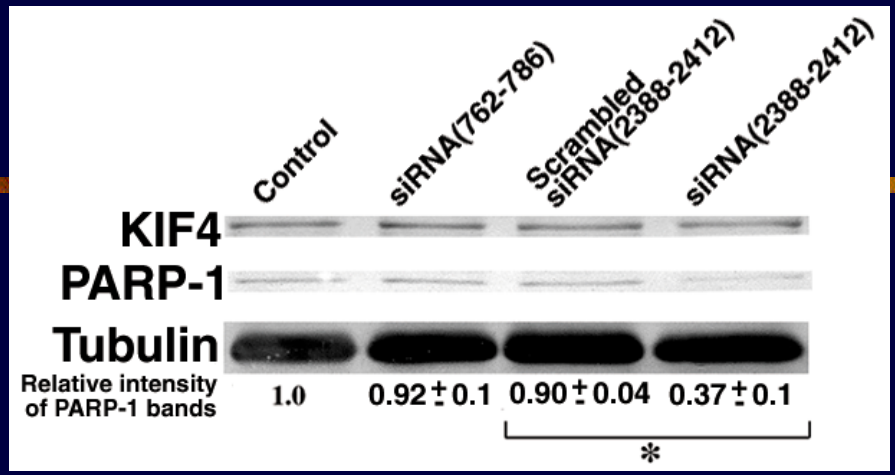
Percentages of apoptic cells

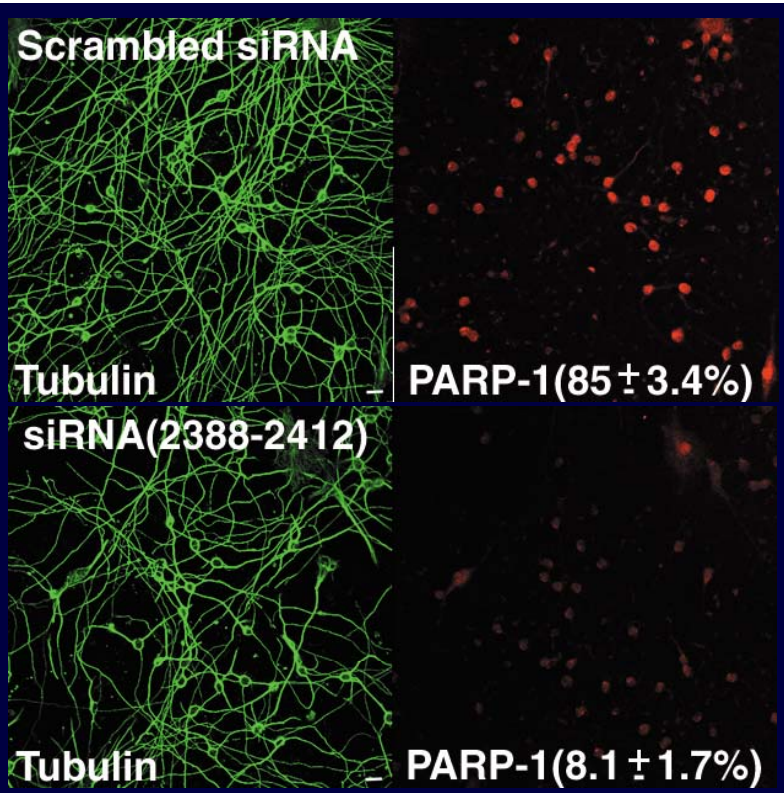


PARP activity of WT and kif4 KO neurons

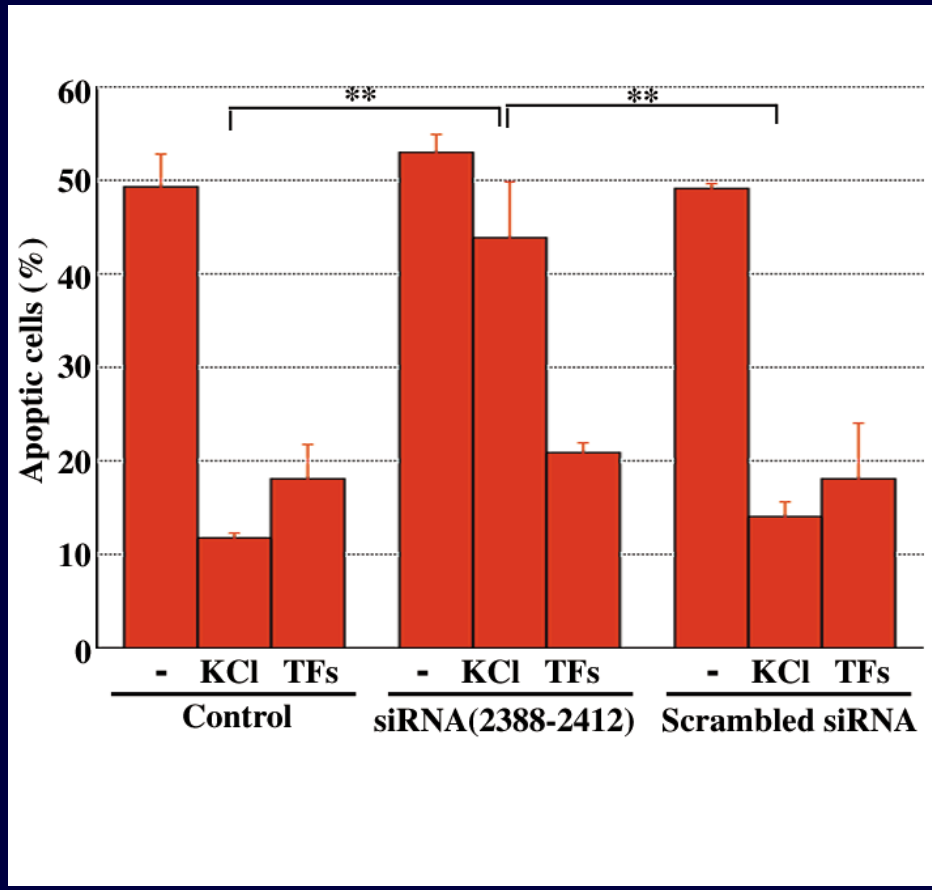


Survival of CGCs (cerebellar granule cells) after knockdown of PARP by RNAi

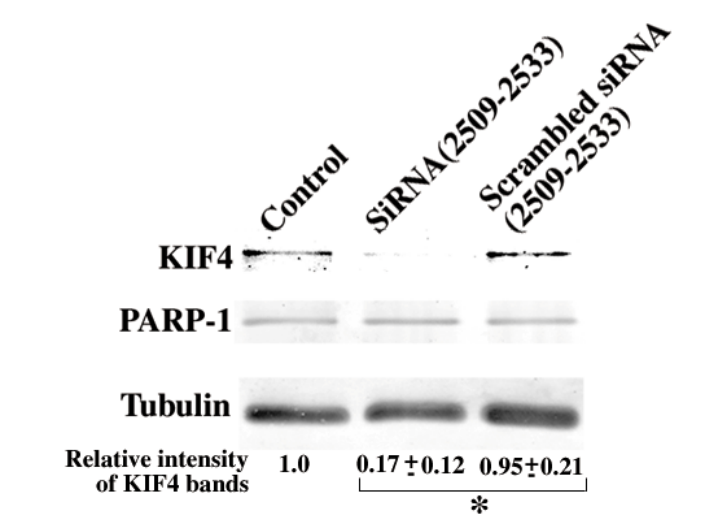




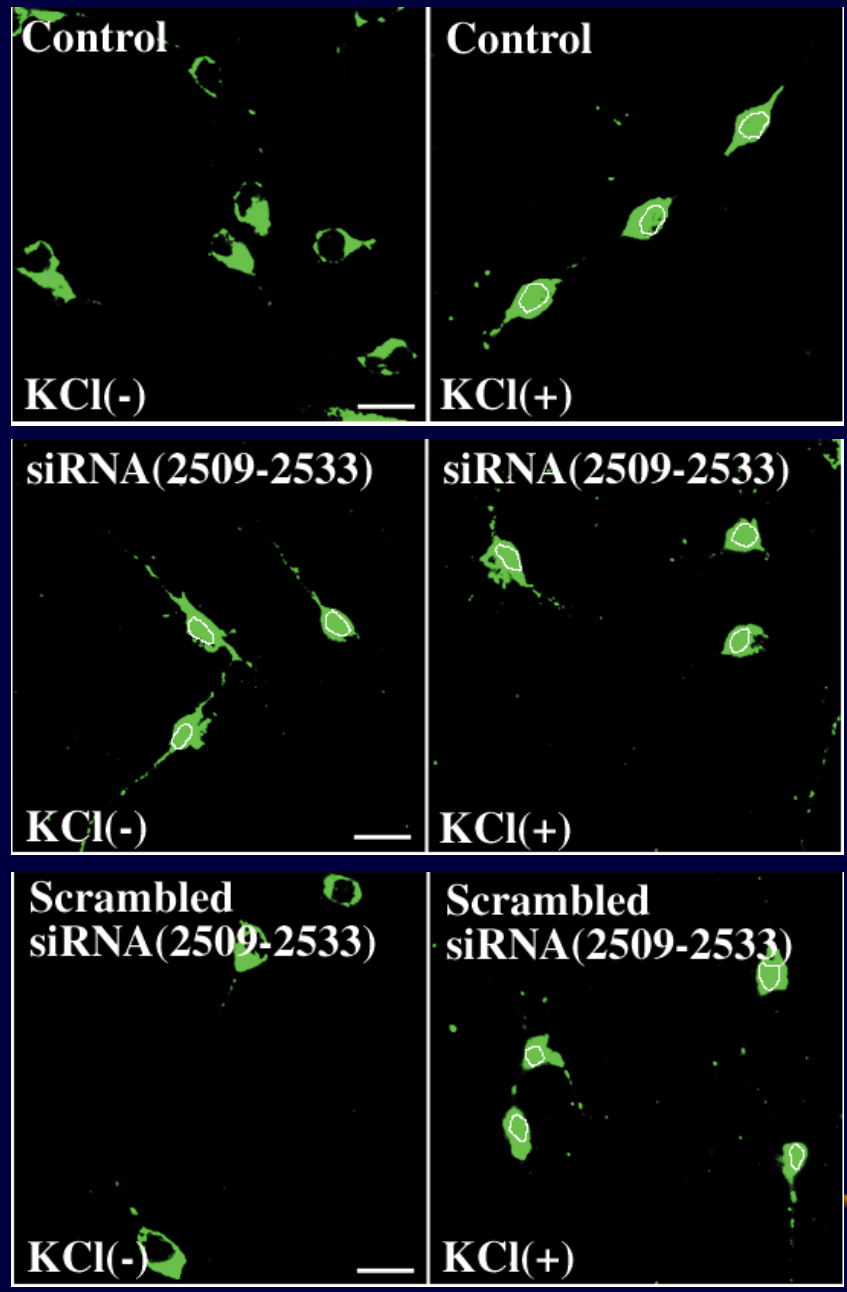
Percentages of apoptic cells



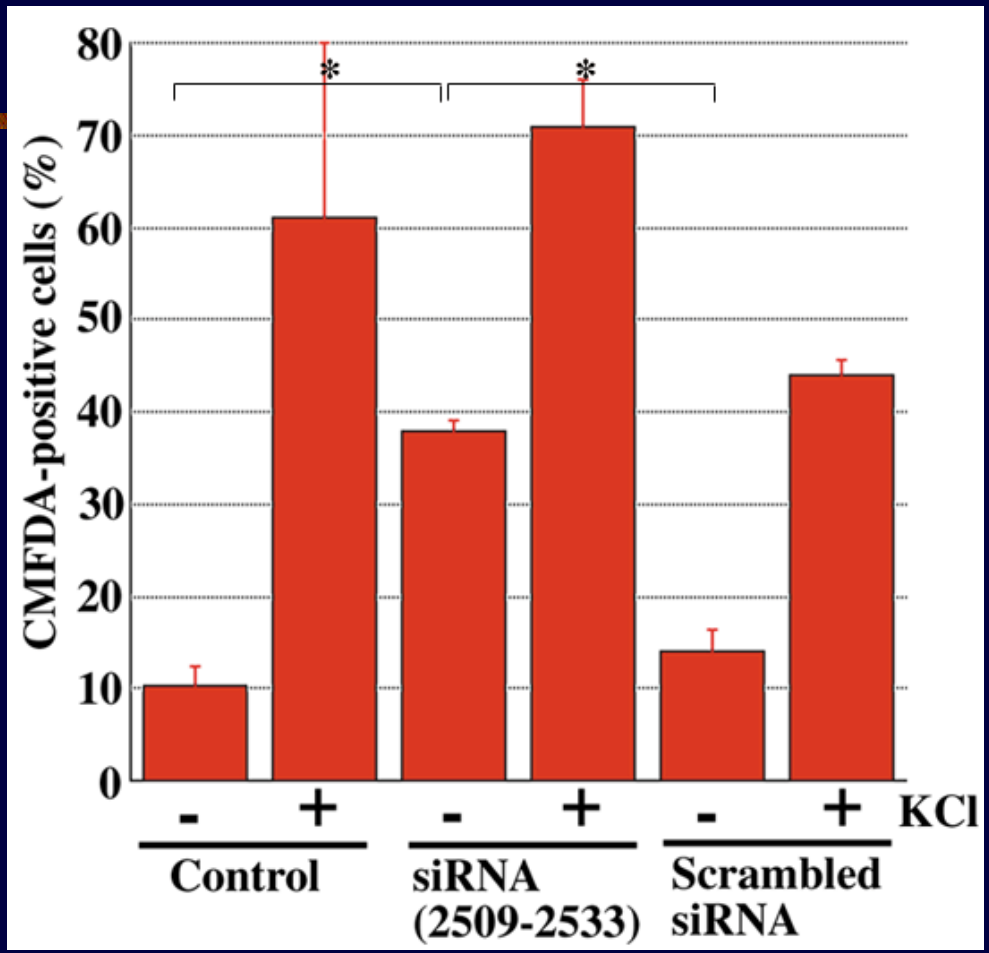
Knockdown of KIF4 in CGCs by RNAi

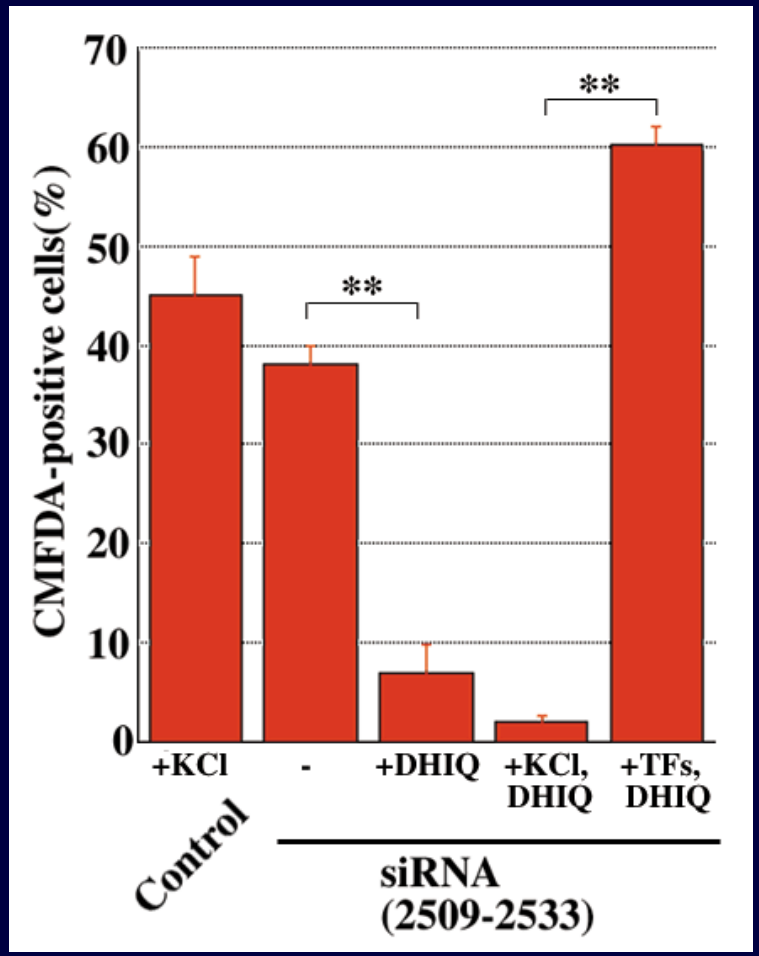


Anti-PAR labeling for CGCs knock down of KIF4

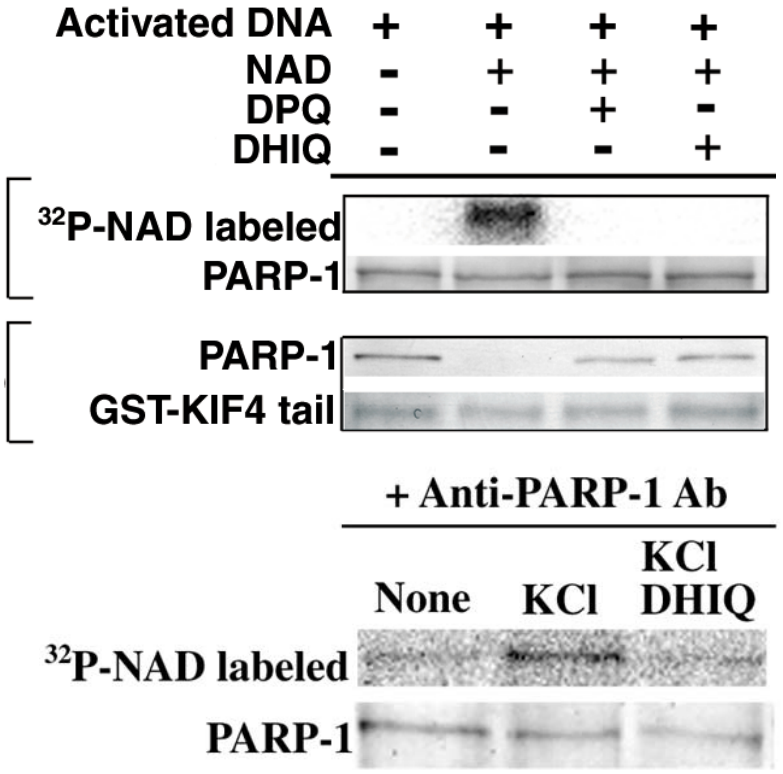


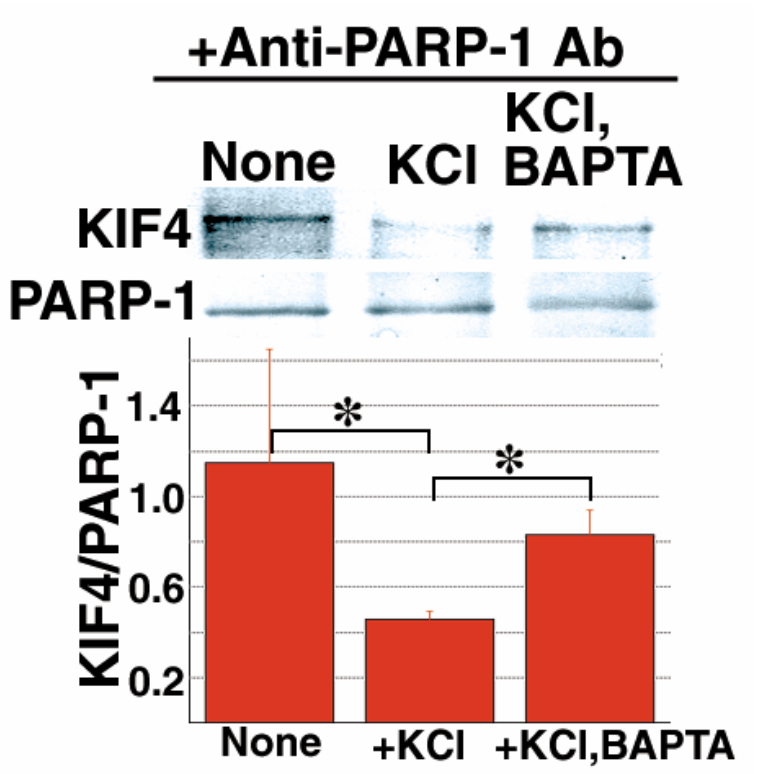
Survival of CGCs after knockdown of KIF4 by RNAi



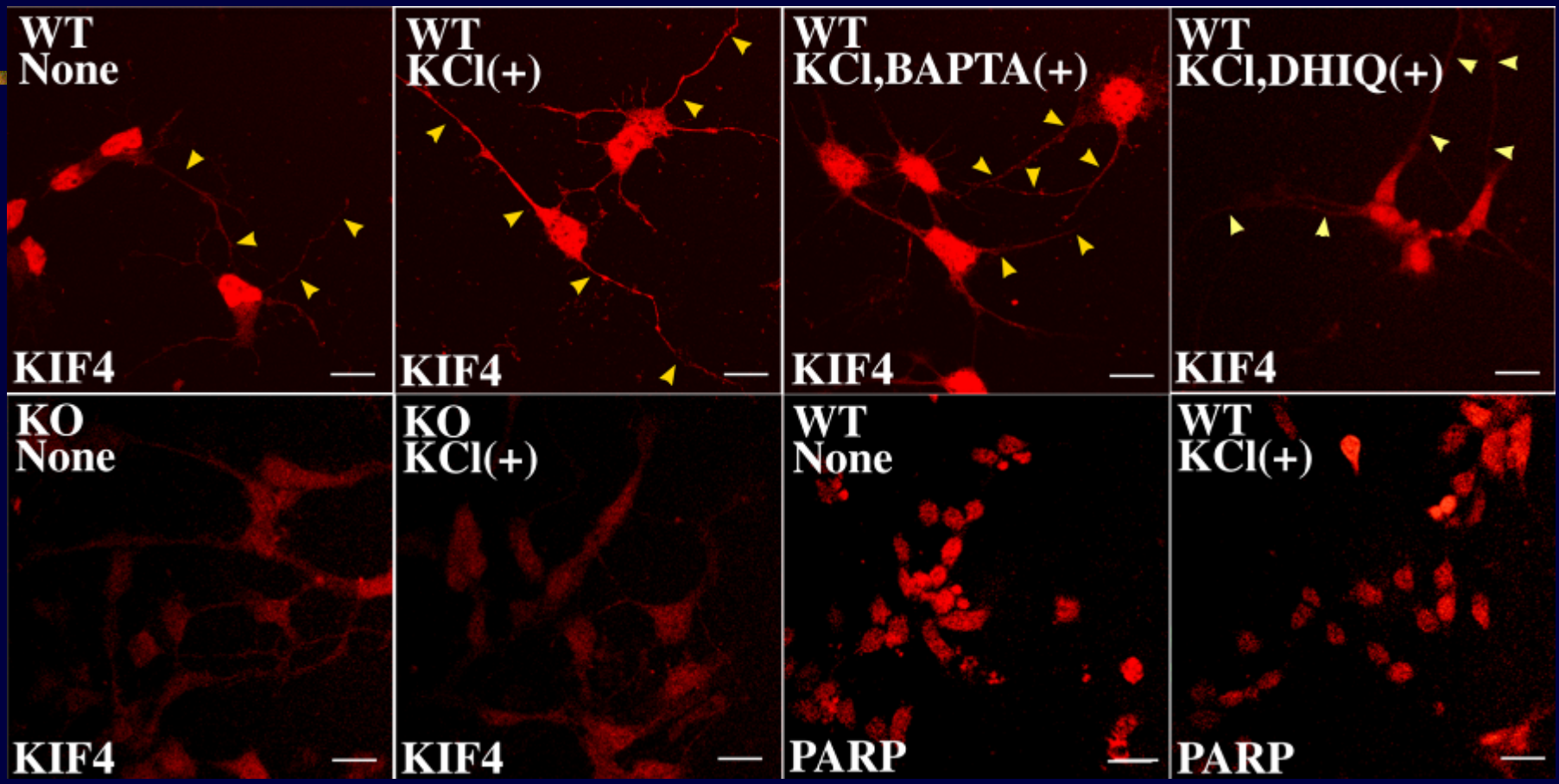


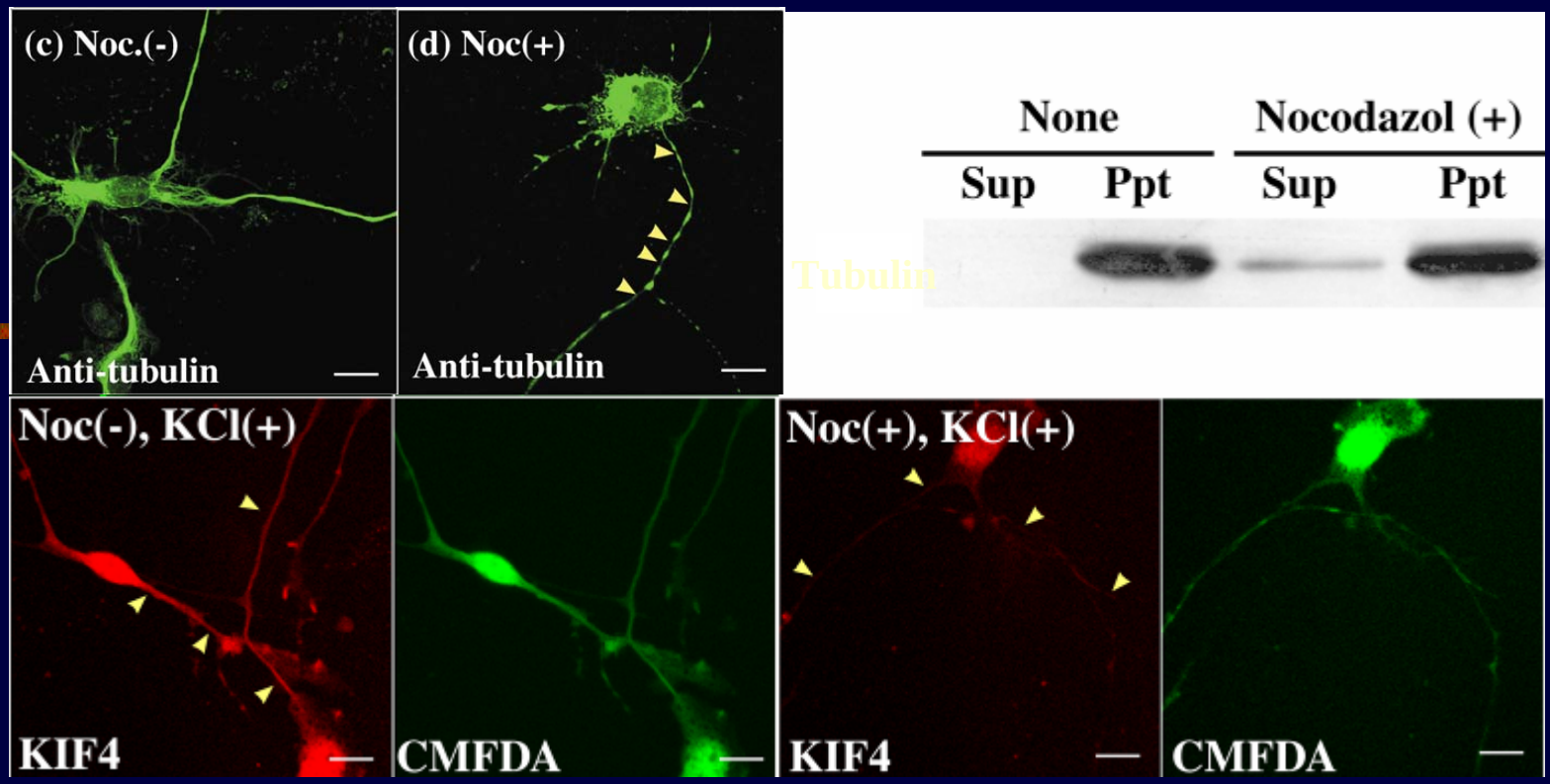
Binding between PARP and KIF4 after depolarization



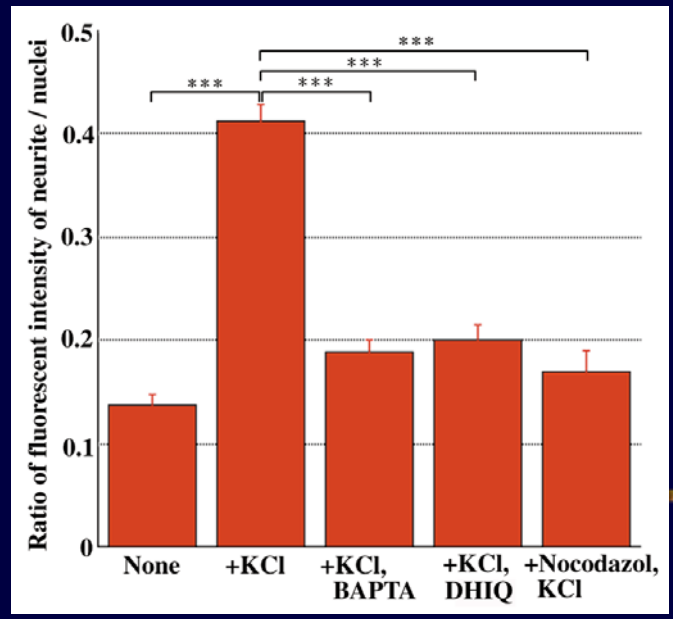


Localization of KIF4 and PARP in ES-derived neurons after depolarization

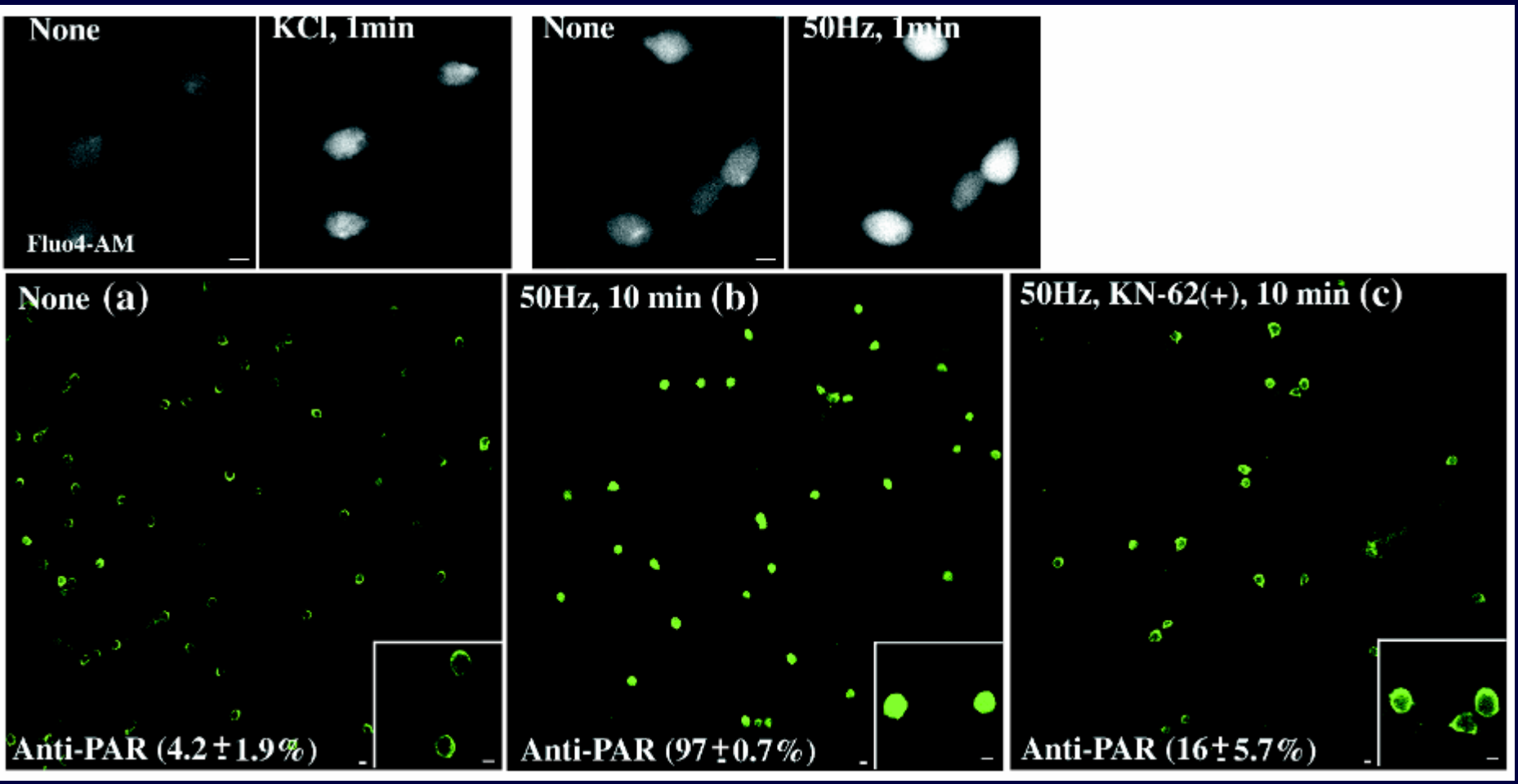


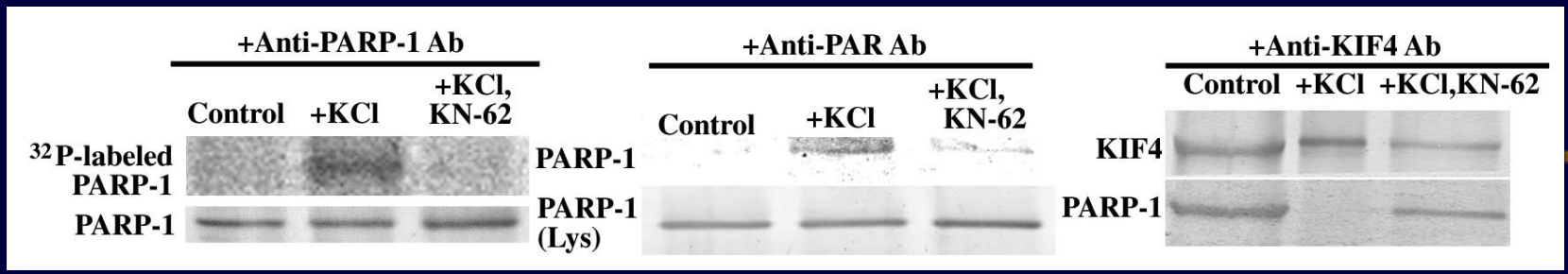


Localization of KIF4 in nocodazol treated neurons

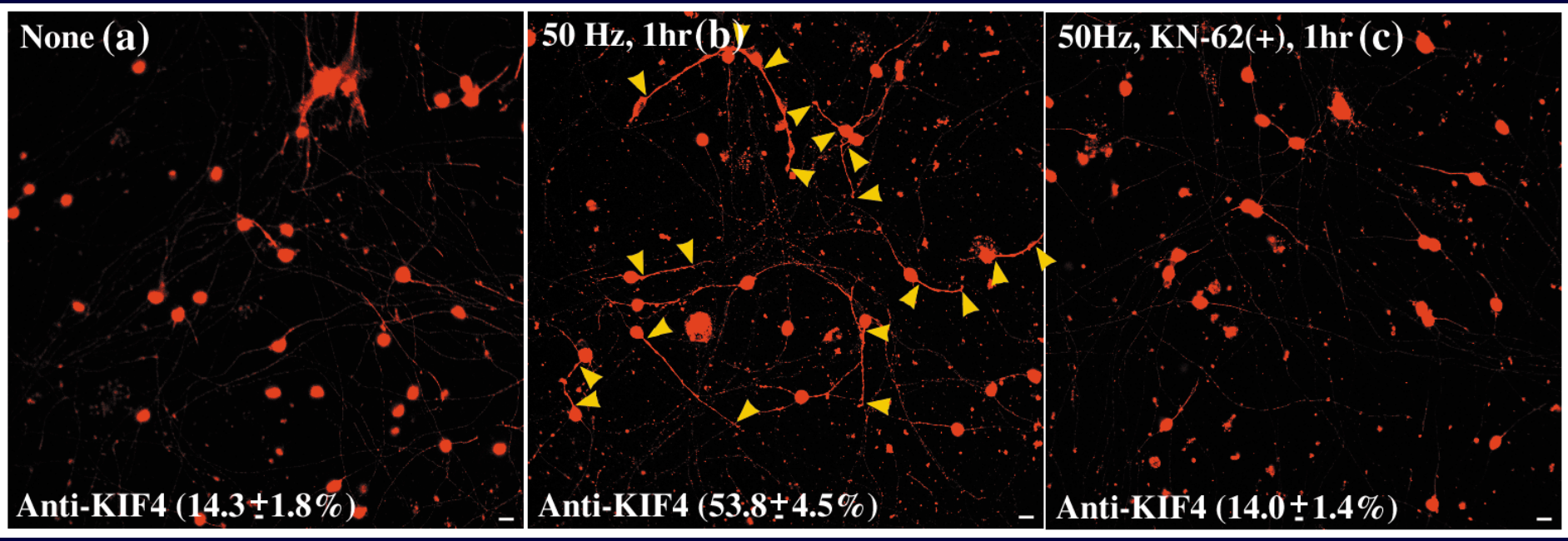


Electrical stimulation applied for CGCs.
(CAMK Ⅱ mediates depolarization-dependent PARP-1 activation)

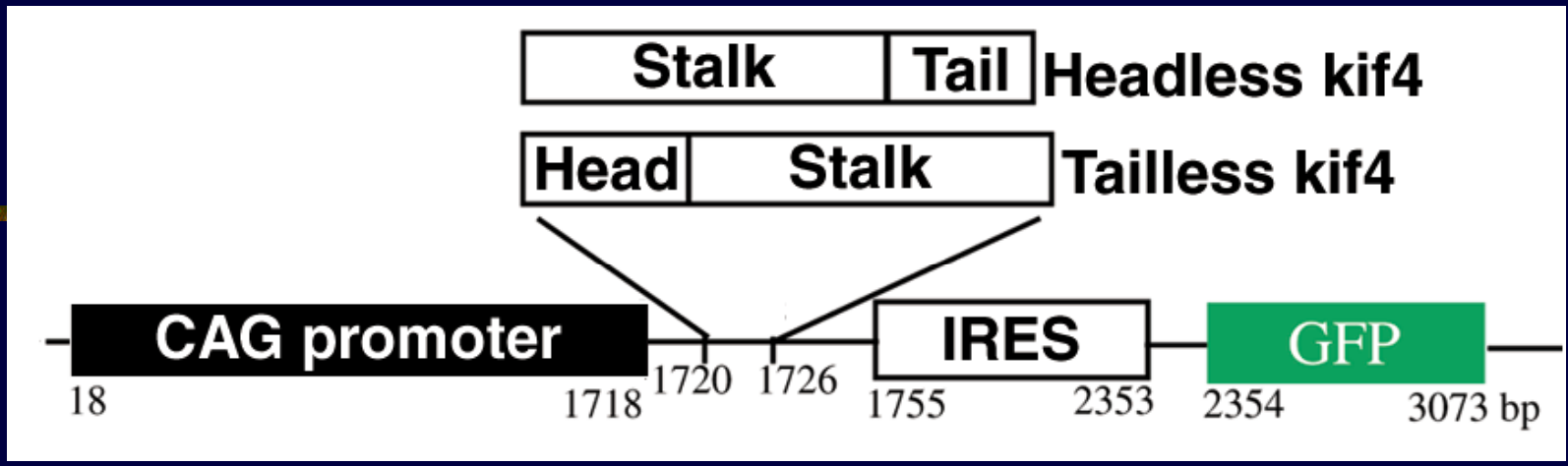


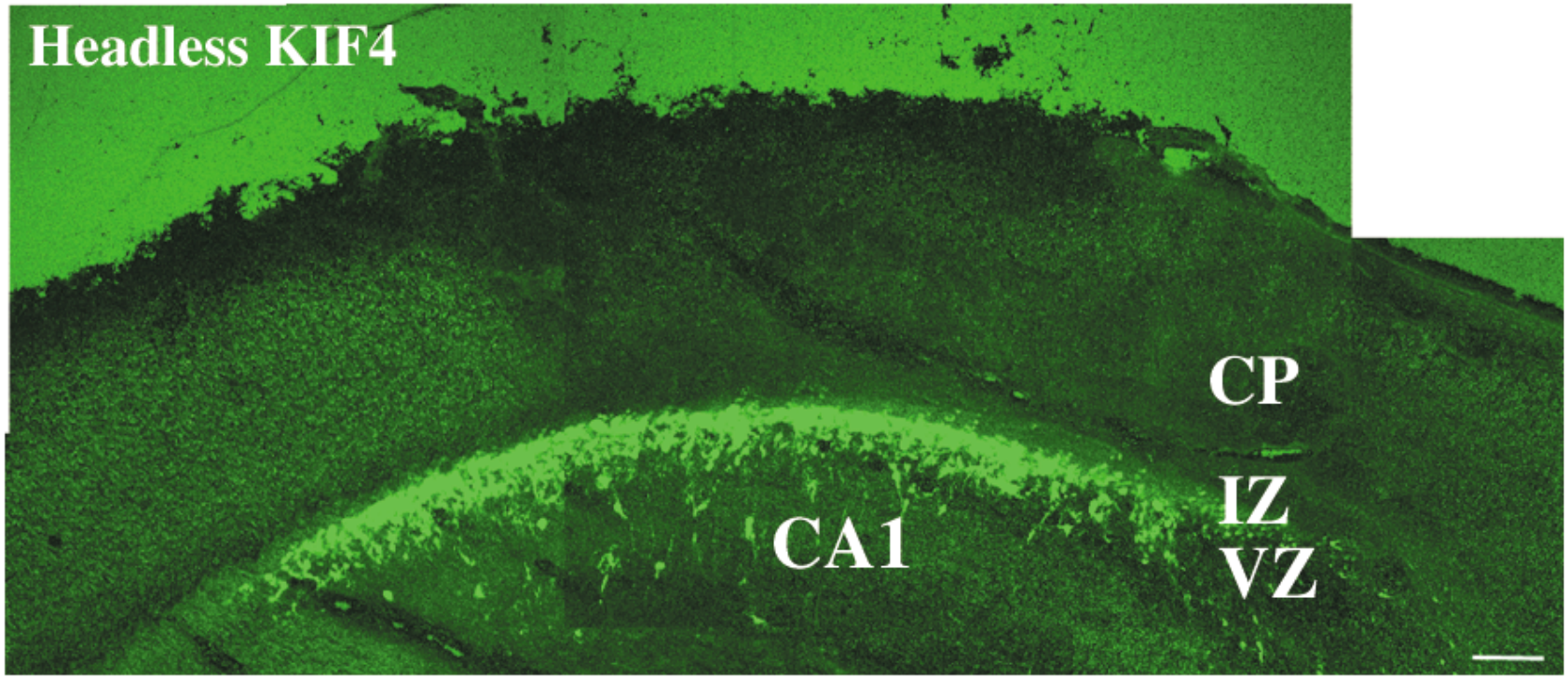


Localization of KIF4 after electrical stimulation in CGCs

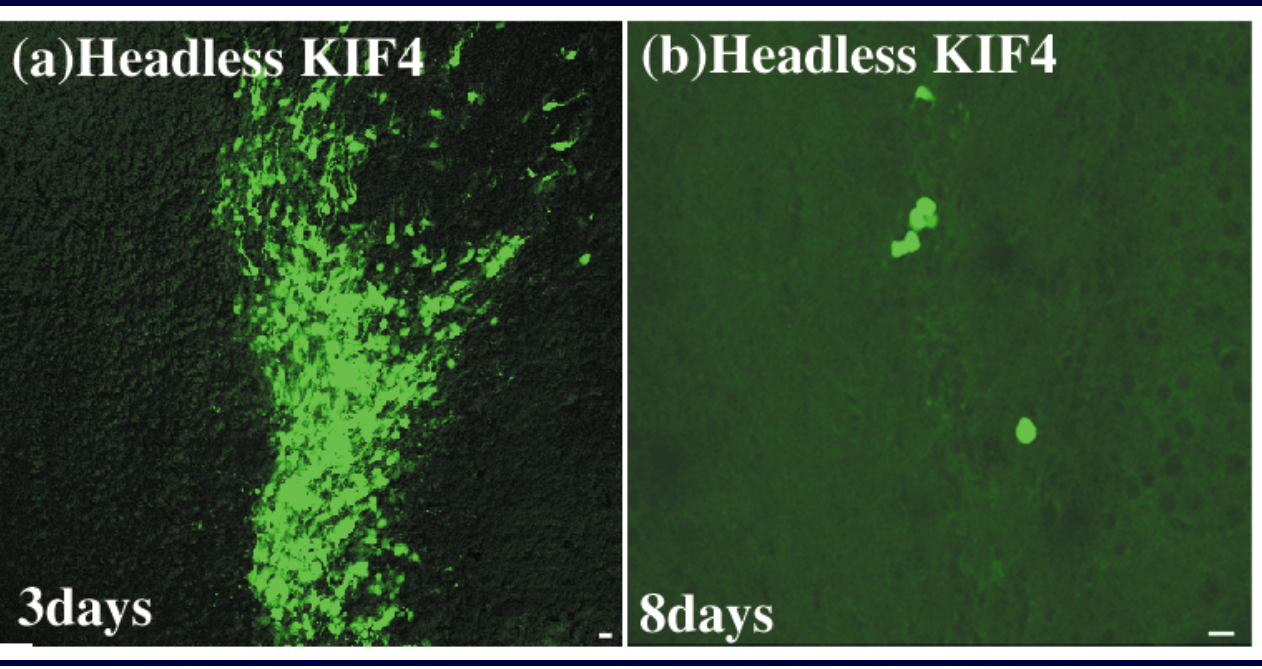


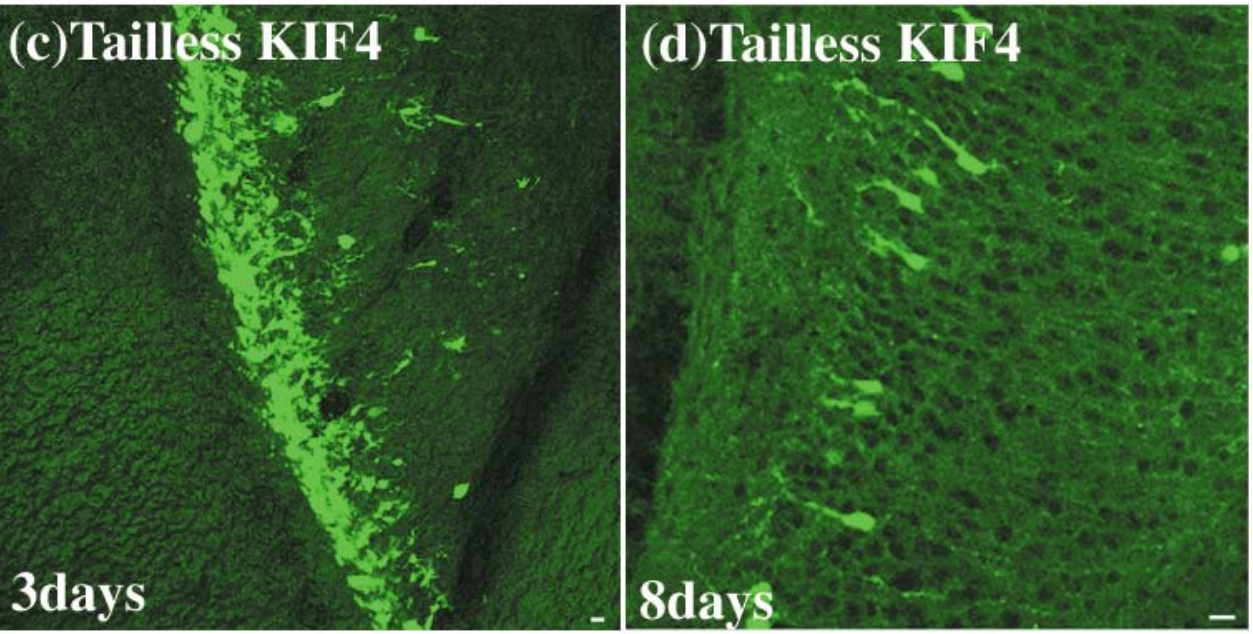
Expression of KIF4 deletion mutants in the brain cortex by in utero electropolation

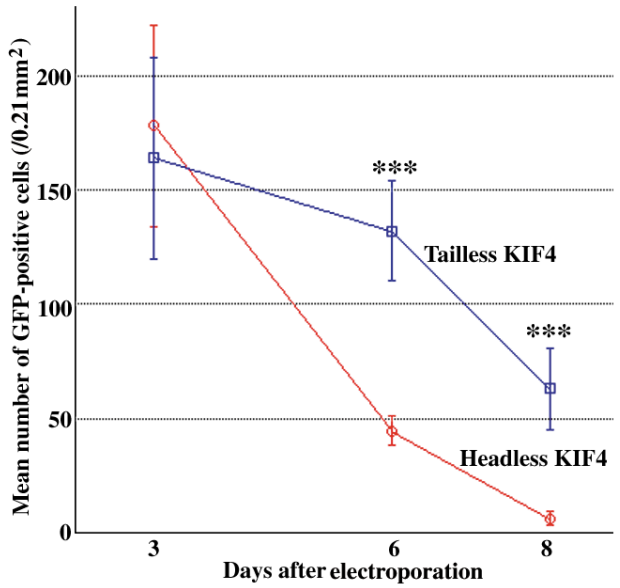




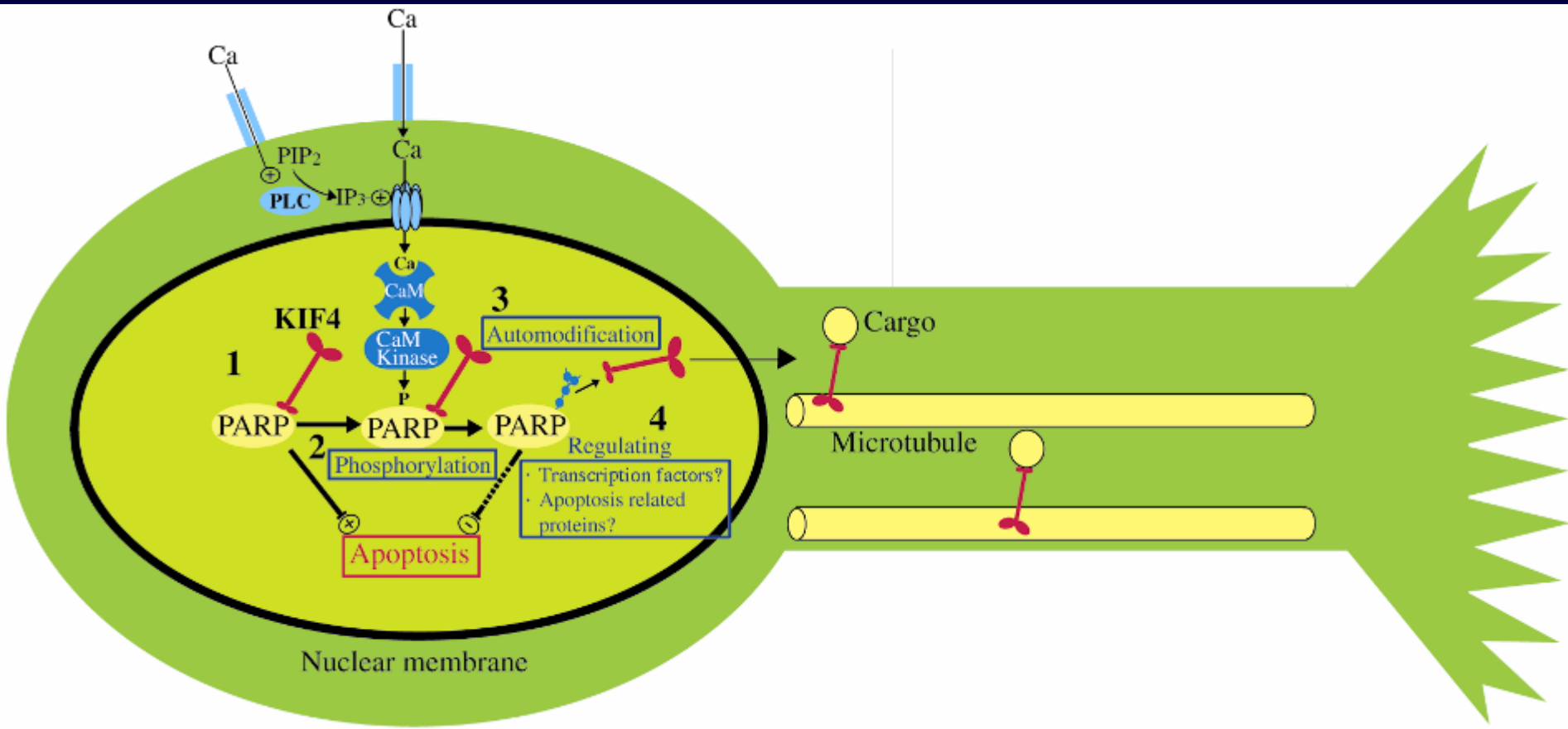
Density of headless-KIF4 and tailless KIF4-positive cells around CA1 region (3 days and 8 days after electropolation)





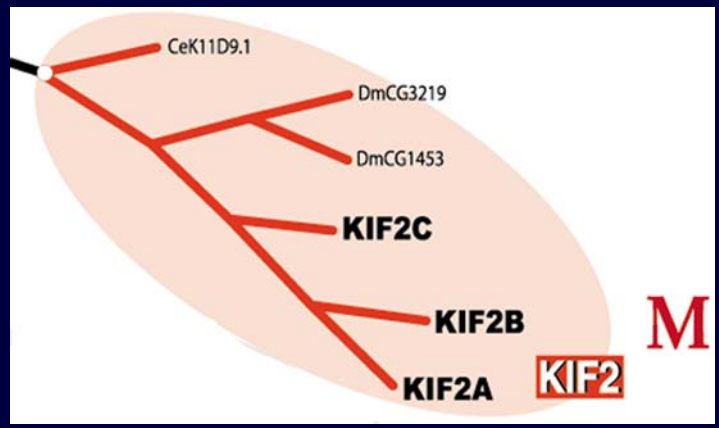


Schematic diagram of the involvement of KIF4 in the regulation of the survival of developing neurons



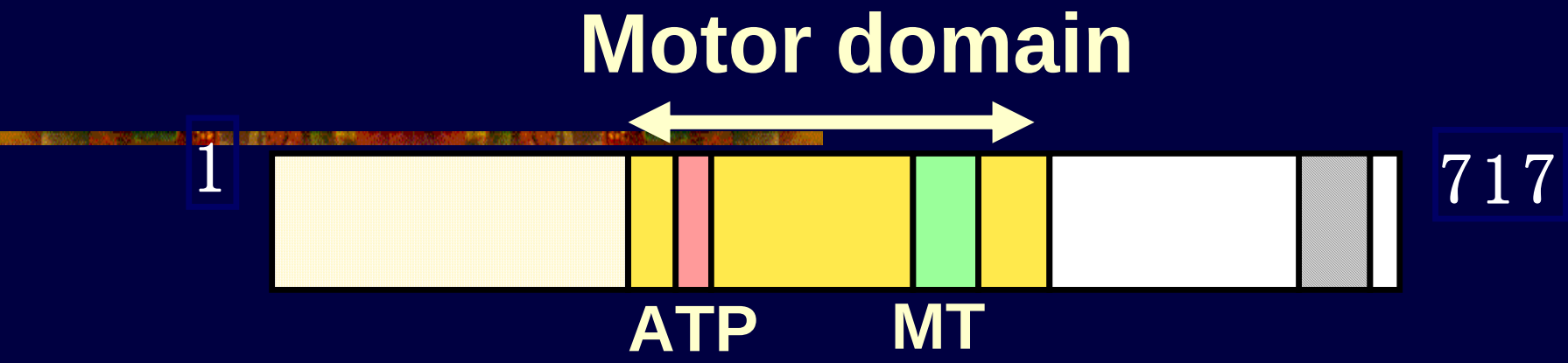
All Family of KIFs

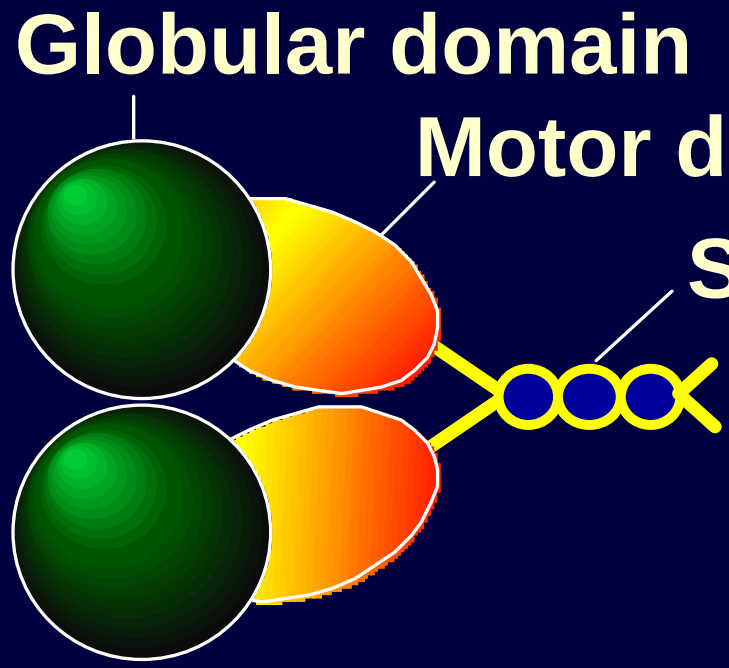
M-kinesins



KIF2C =MCAK
XKCM1, XKIF2 in *Xenopus*

KIF2A

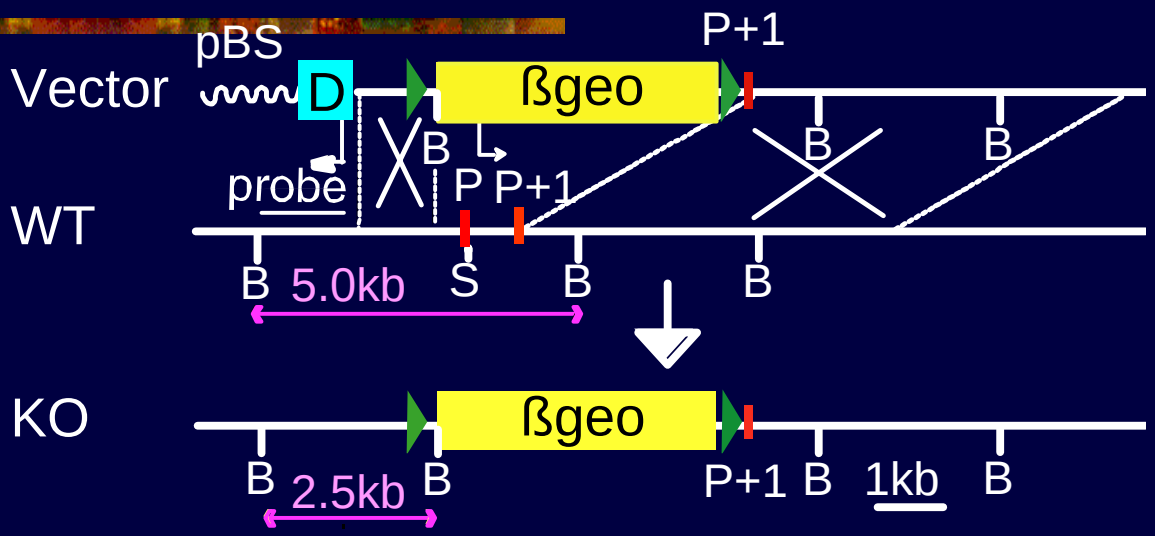




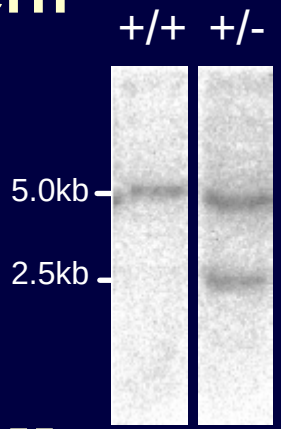
Abundant in juvenile brain

Generation of *kif2a*$^{-/-}$ mice

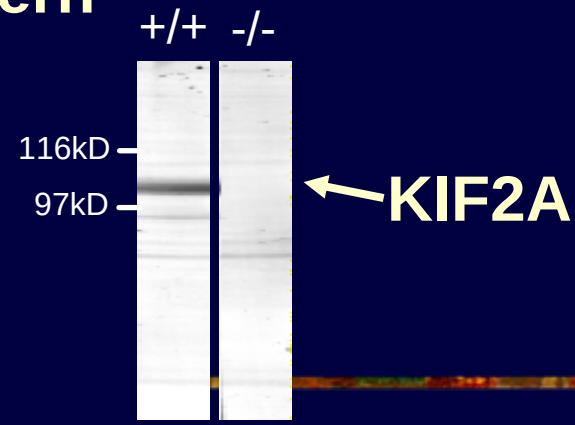
Construction



Southern



Western



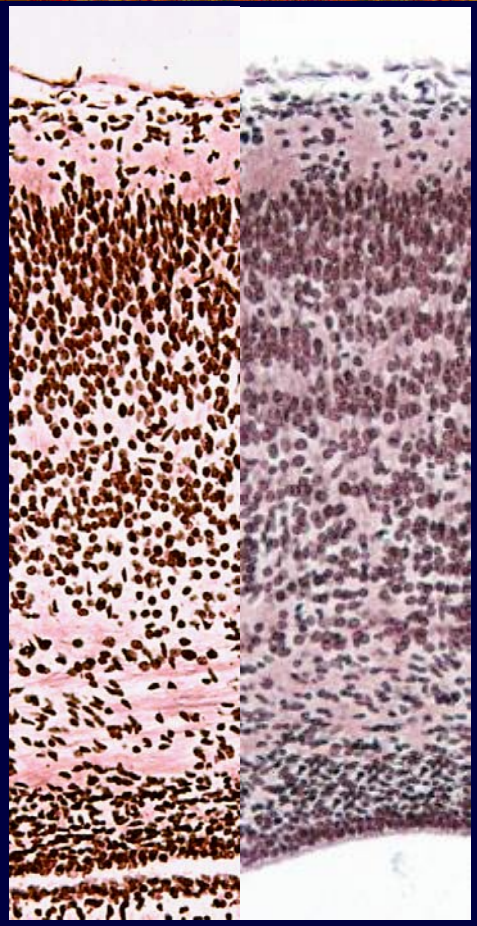
Homma et al. Cell 114:229—,2003

kif2a$^{-/-}$ mice

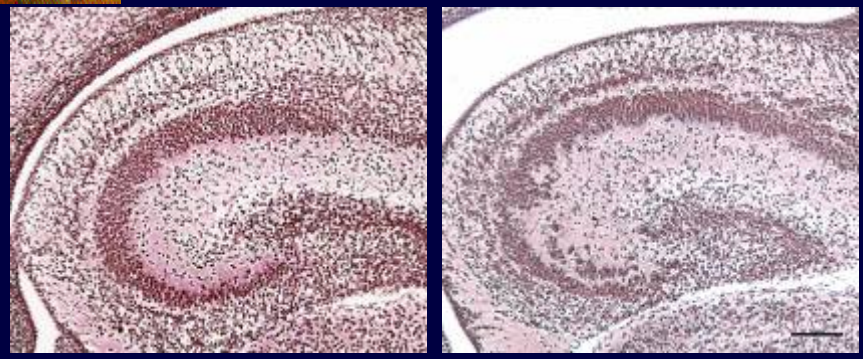
***kif2a*$^{-/-}$ mice died in the day without suckling milk.**

Laminary defects in *kif2a*$^{-/-}$ brain

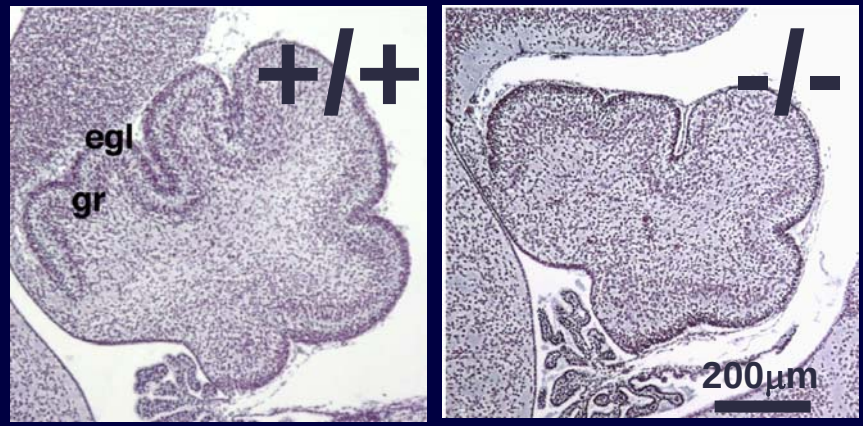
Cortex



Hippocampus

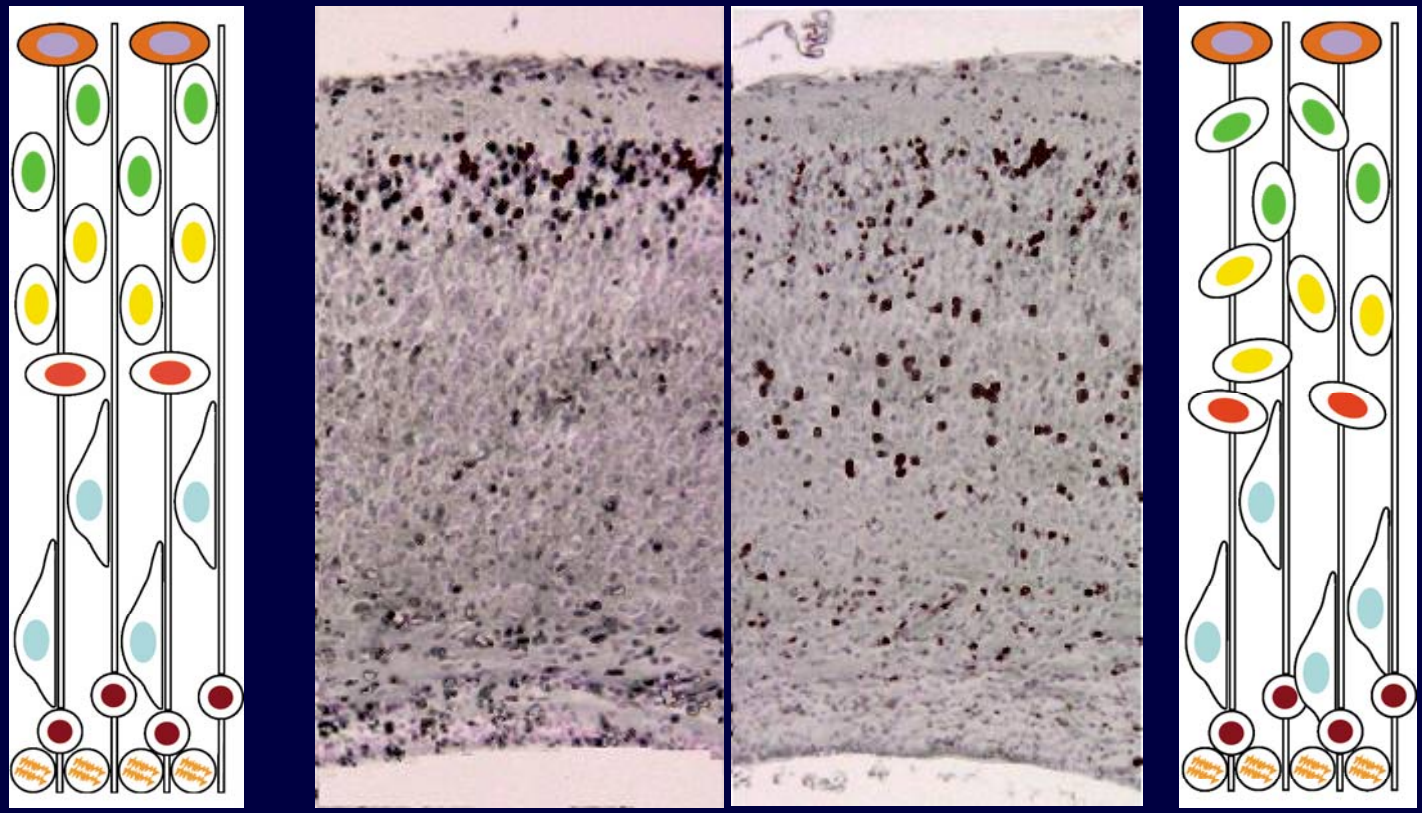


Cerebellum

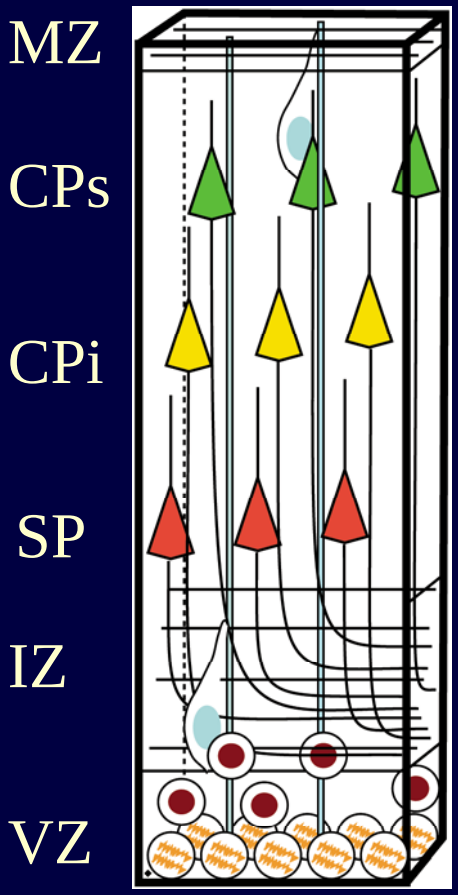


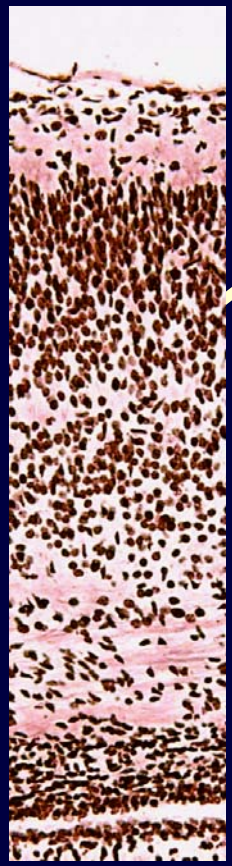
Migratory defects in *kif2a*$^{-/-}$ brain

+/+ E14+/+ E14-/- -/-

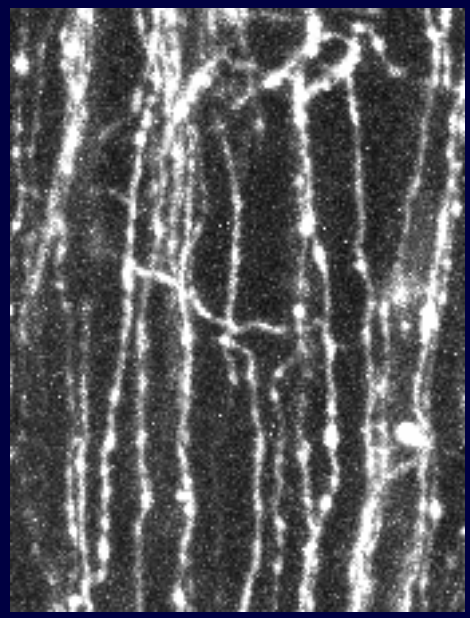


Axon branching abnormality in *kif2a*$^{-/-}$ cortex

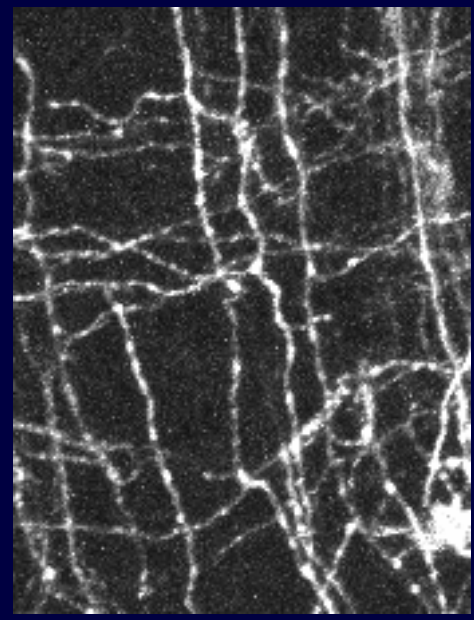




+/+

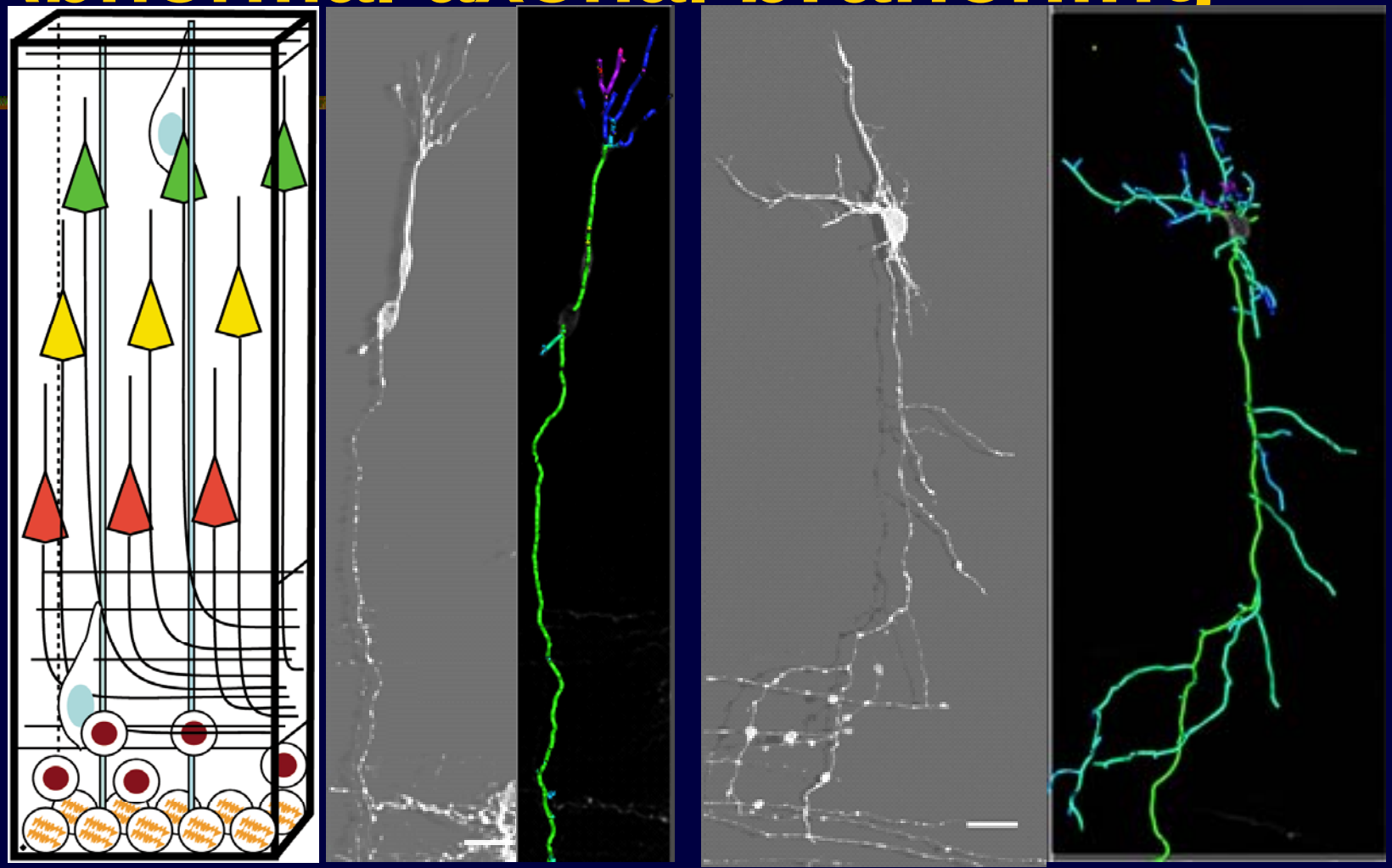


-/-

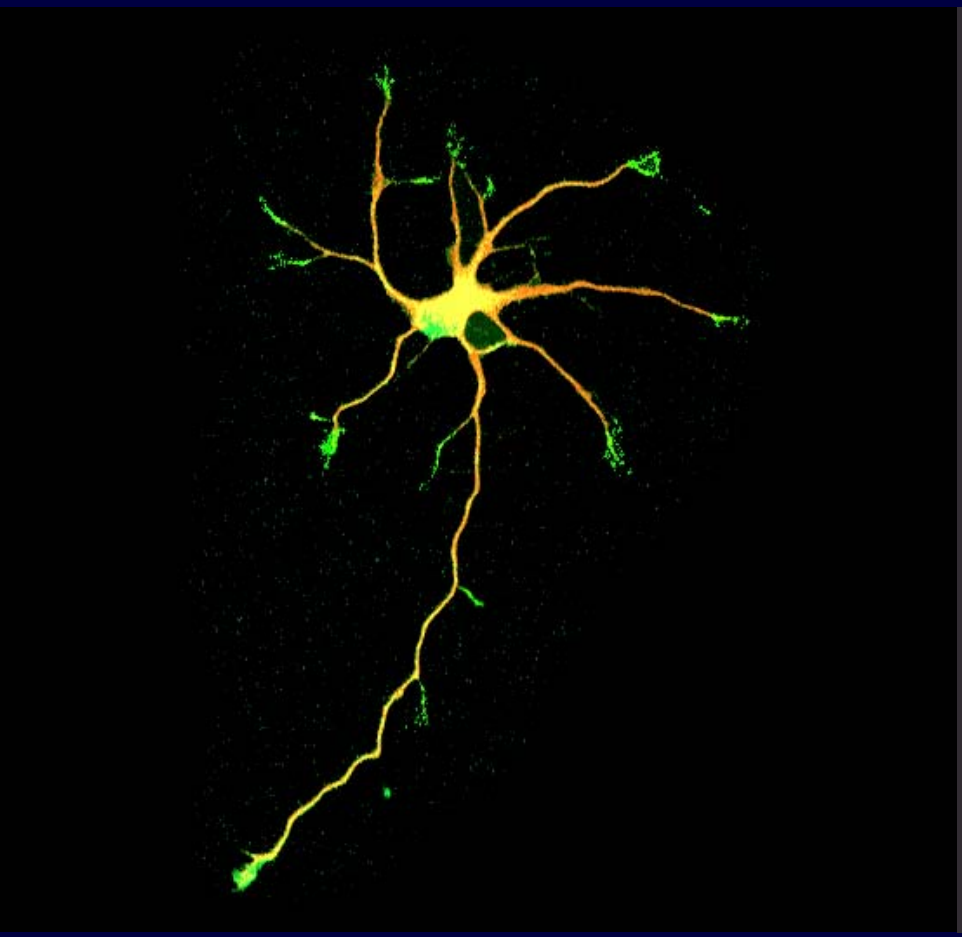


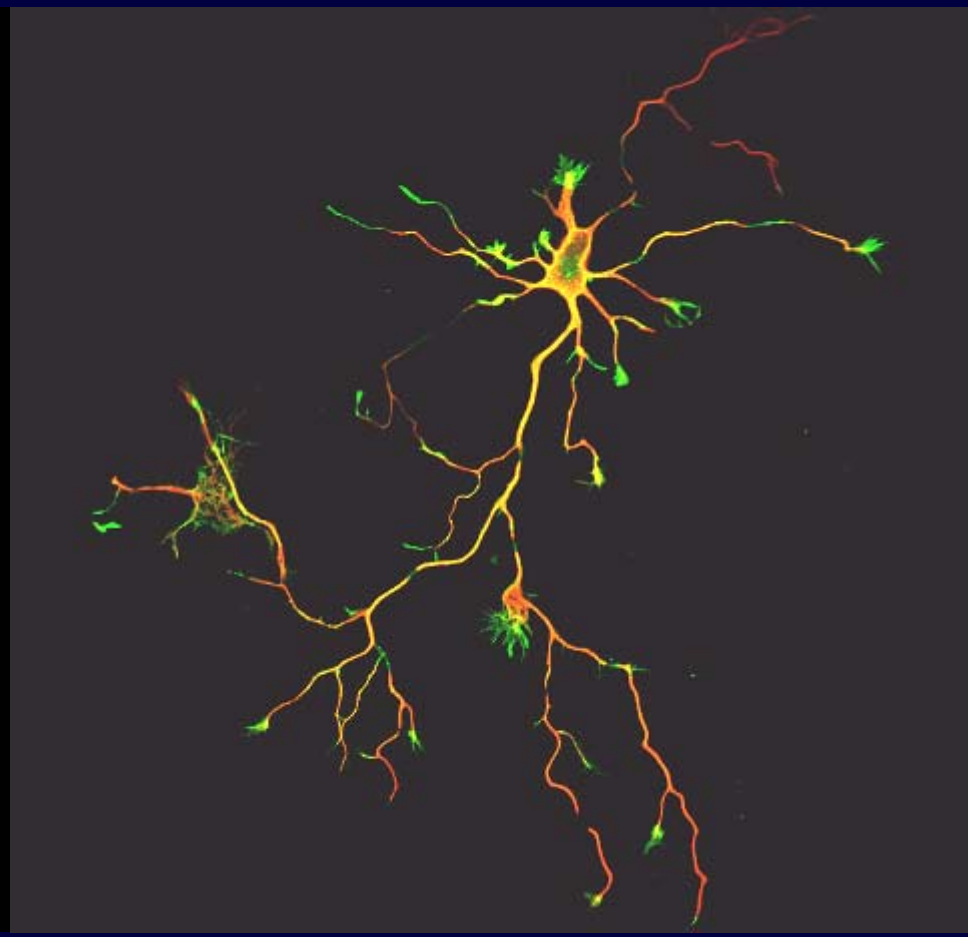
More horizontal neurites are observed in *kif2a*$^{-/-}$ cortex than in *kif2a*$^{+/+}$ cortex.

Abnormal axonal branching



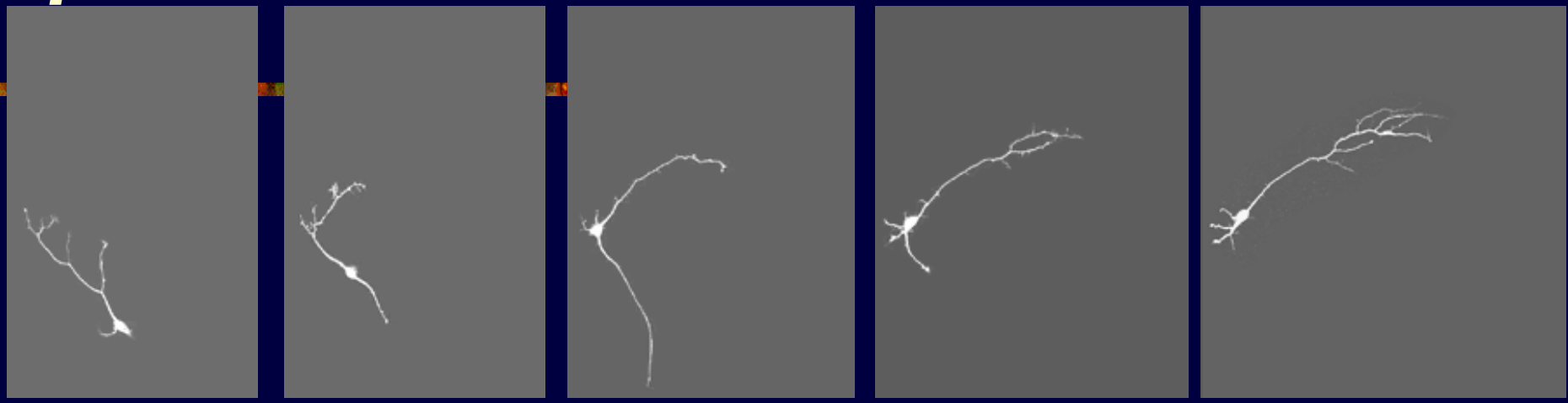
Abnormal arborization in *kif2a*$^{-/-}$ hippocampal neuron



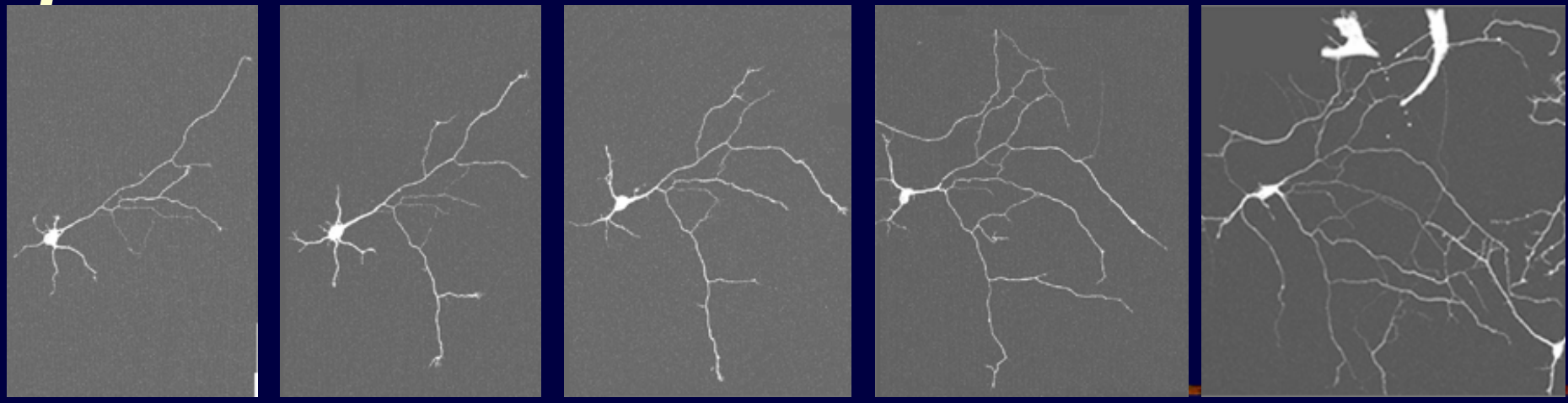


Time-lapse Imaging

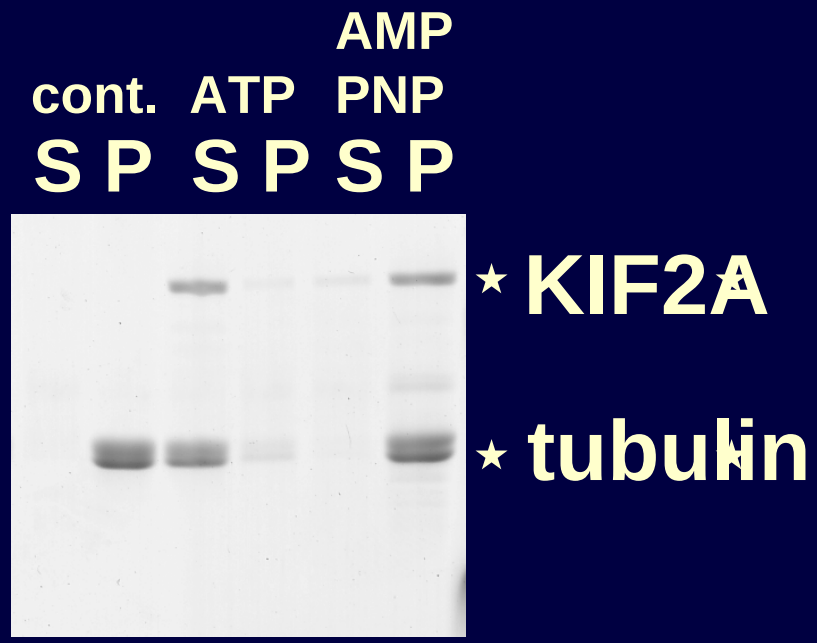
+/+



-/-



KIF2A depolymerizes MT *in vitro*

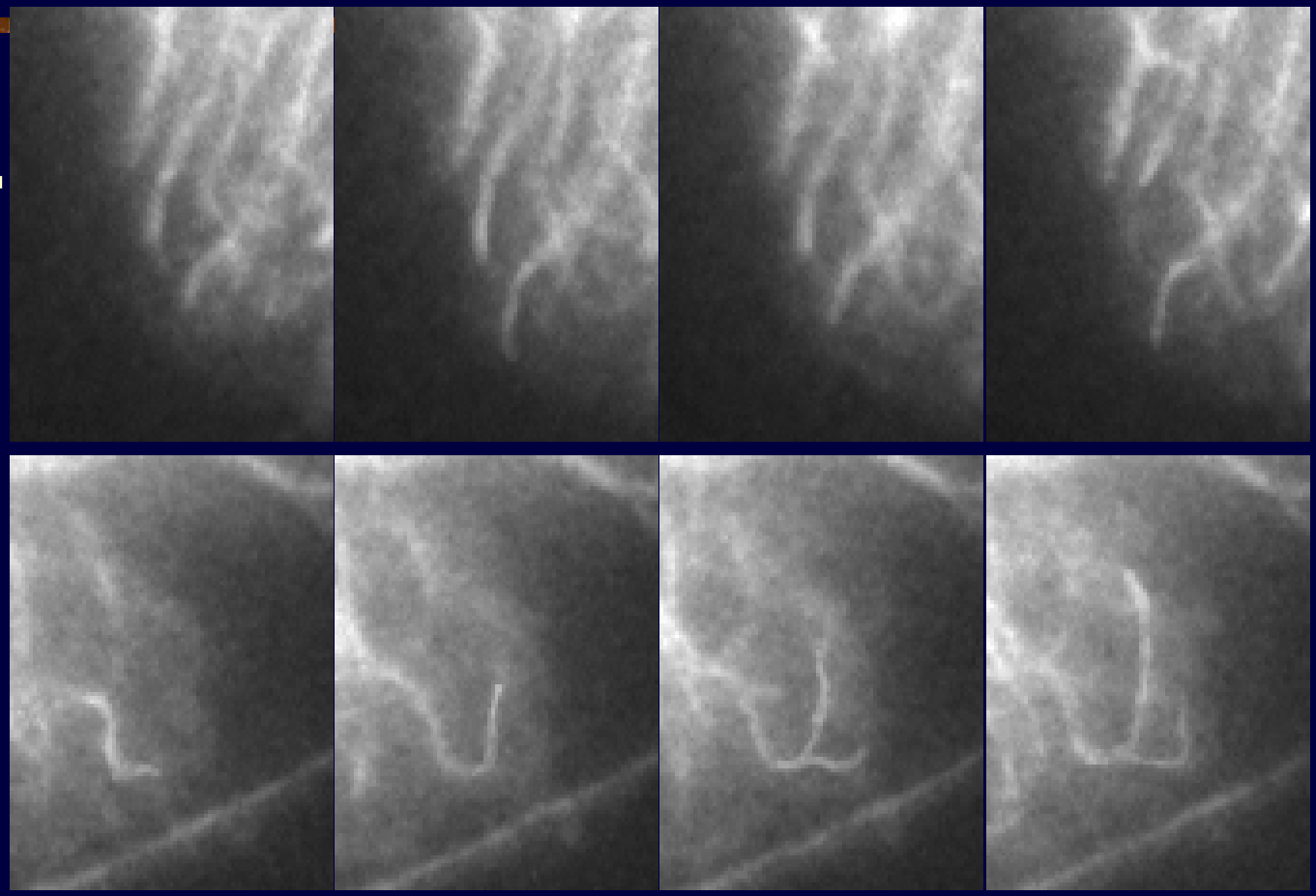


Tubulins are recovered from the supernatant in the presence of ATP as a result of MT depolymerization by KIF2A.

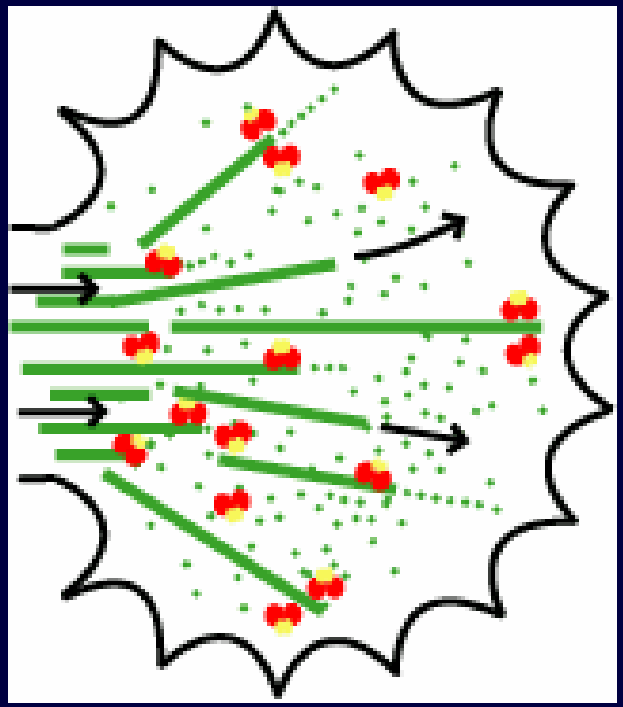
Movement of individual MTs

+/+

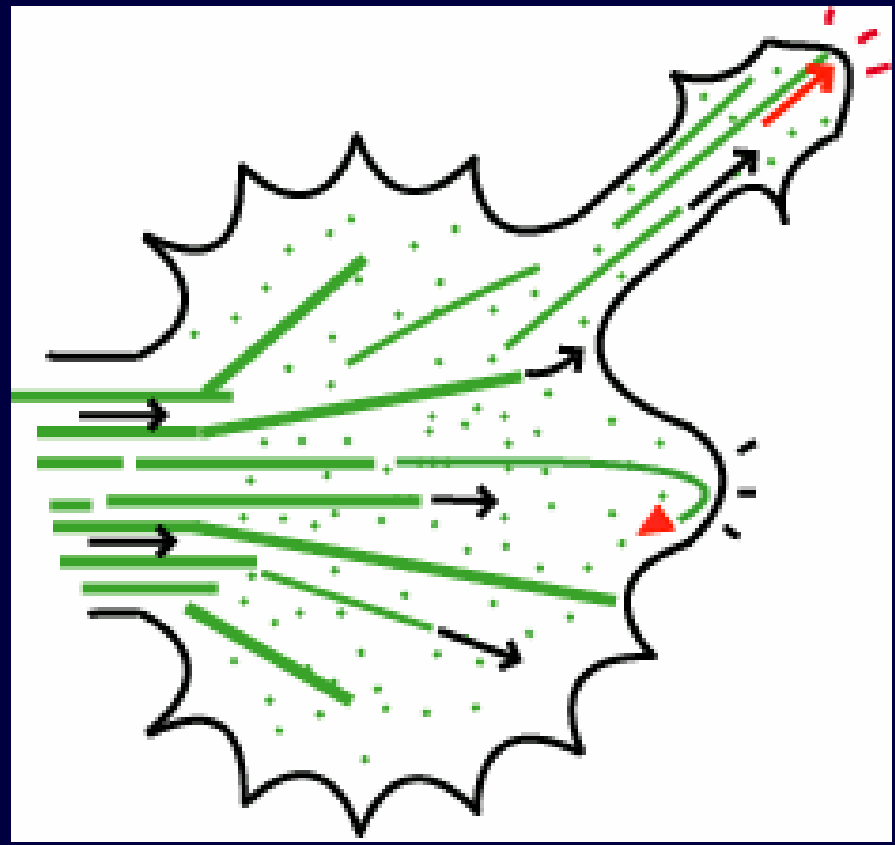
-/-



KIF2A function in growth cone



+/+



-/-