Different intracellular localization of STAM adaptor proteins in neurons

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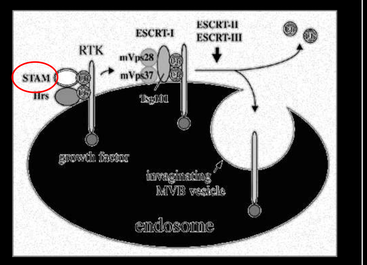
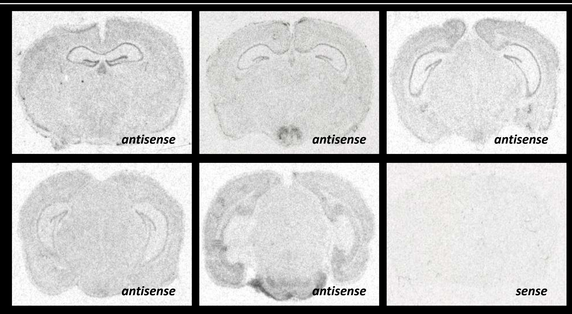
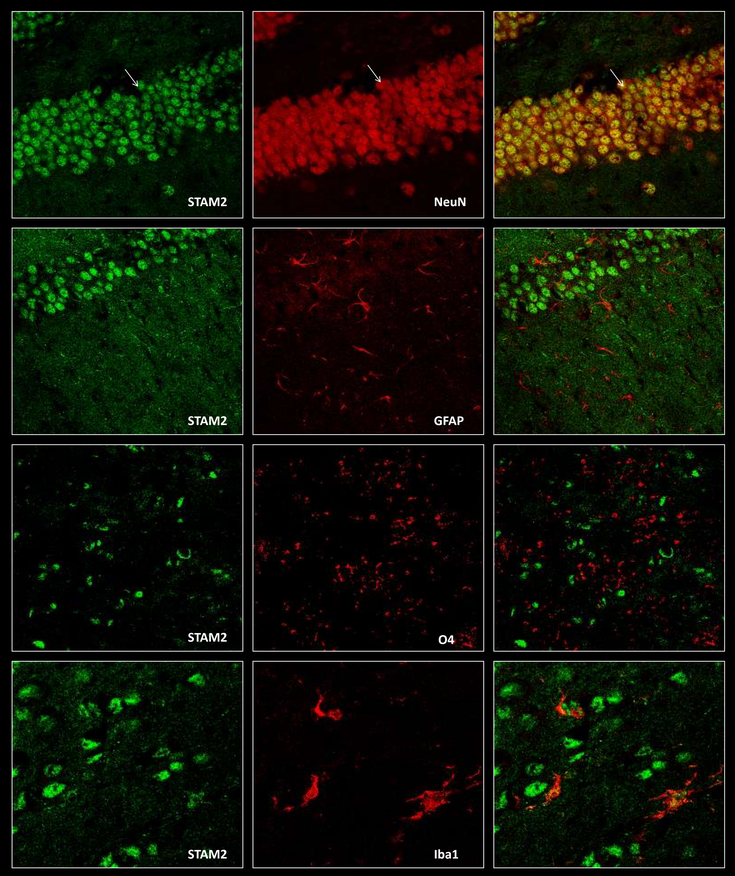
STAM1 and STAM2 (Signal transducing adaptor molecule) are phosphotyrosine proteins, members of the endosome associated complex ESCRT-0, involved in sorting of mono-ubiquitinated endosomal cargo for degradation in the lysosome. Apart the suggested role in enodosomal trafficking, STAM possible roles are in cytokine signaling as well. STAM are phosphorylated on tyrosine upon stimulation with a variety of cytokines and growth factors. They are associated with JAK2 and JAK3, and are involved in the regulation of intracellular signal transduction for DNA synthesis and c-myc induction mediated by IL-2 and GM-CSF.

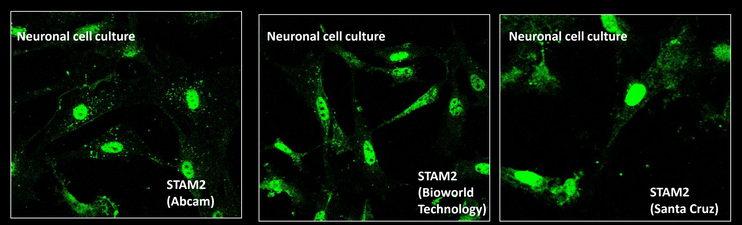




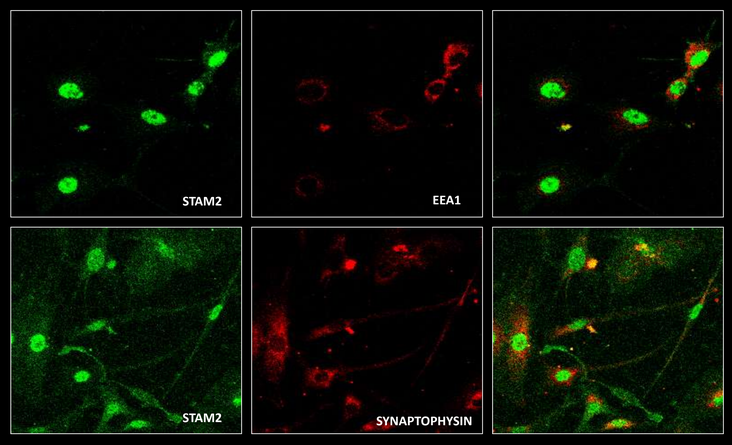
To to determine *Stam2* expression pattern in the CNS of the mice.*in situ* RNA hybridization sing radioactive (S35) labelled probe was made.



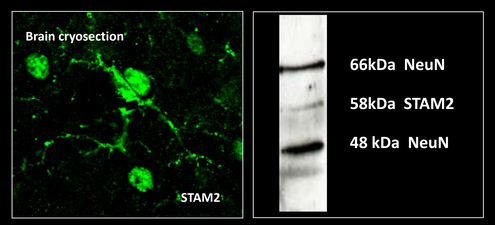
In order to clarify which cell types highly expressed STAM2, we found colocalization of STAM2 proteins with neuronal marker NeuN, but no colocalization (or very weak signal) with GFAP, O4 and Iba1 as astrocyte ,oligodendrocyte and microglia cell marker.



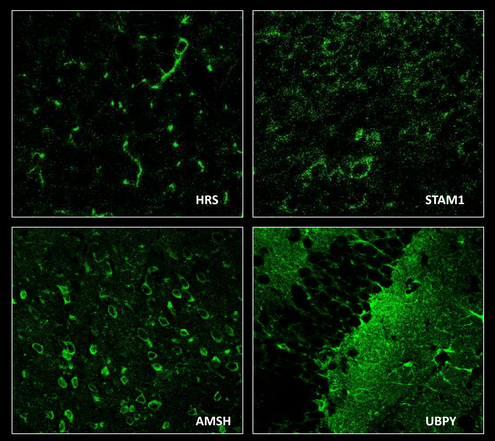
Surprisingly, we also found homogenous signal in the neuron nuclei using immunohistochemical staining method with three different commercial antibodies (cryosections and neuronal primary cell cultures). These results were verified by western blot analysis on the neuronal nuclear protein fraction.



Double staining with STAM2/EEA1 and STAM2/synaptophysin antibody confirmed that STAM2 was localized in the punctuate structures positive for EEA1 (early endosome marker), but also positive for synaptophysin (synaptic vesicle protein).



Western blot analysis on the neuronal nuclear protein fraction



To examine the nuclear expression of ESCRT-0 members (STAMs and Hrs) in the brain we performed immunohistochemical analysis with the other STAM isoform, STAM1, and with Hrs, as well with STAM2- associated molecule, AMSH and UBPY. These proteins are not localized in the nuclei.

***<u>CONCLUSION:</u>** As several proteins involved in ESCRT complex play additional role in the cell nucleus, these results suggest that endocytic and signaling machineries are more intimately connected than previously thought and indicate that some proteins may play a dual role in sorting proteins for lysosomal degradation and in regulating nuclear gene expression. One of these proteins with possible dual role might be STAM2.*