

Table 1-1. Polyglutamine Disease Proteins

| Disease | Normal Repeats | Mutant Repeats | Protein | Normal Protein Distribution | Reference |
|----------------|-----------------------|-----------------------|--|------------------------------------|---|
| DRPLA | 3-36 | 49-88 | Atrophin | Nuclear & Cytoplasmic | Becher et al., 1998 |
| HD | 11-35 | 36-120 | Huntingtin | Cytoplasmic | Huntington's Disease Collaborative Research Group 1993 |
| SBMA | 9-36 | 38-62 | Androgen Receptor | Nuclear | Butler et al., 1998 |
| SCA-1 | 6-39 | 40-83 | Ataxin-1 | Nuclear | Skinner et al., 1997 |
| SCA-2 | 14-32 | 33-77 | Ataxin-2 | Cytoplasmic | Pang et al., 1997 |
| SCA-3 | 12-40 | 55-86 | Ataxin-3 MJD1 | Cytoplasmic | Ikeda et al., 1996 |
| SCA-6 | 4-18 | 21-30 | P/Q Ca²⁺ Channel | Membrane | Ishikawa et al., 2000 |
| SCA-7 | 7-17 | 38-200 | Ataxin-7 | Nuclear | Holmberg et al., 1998 |
| SCA-17 | 25-42 | 47-55 | TBP | Nuclear | Nakamura et al., 2001 |

Abbreviations: DRPLA, dentatorubral-palidolusian atrophy; HD, Huntington's disease; SBMA, spinal and bulbar muscular atrophy; SCA, spinocerebellar ataxia; MJD, Machado-Joseph disease; TBP, TATA binding protein.

Figure 1-1. Model of the Ubiquitin-Proteasome System Pathway

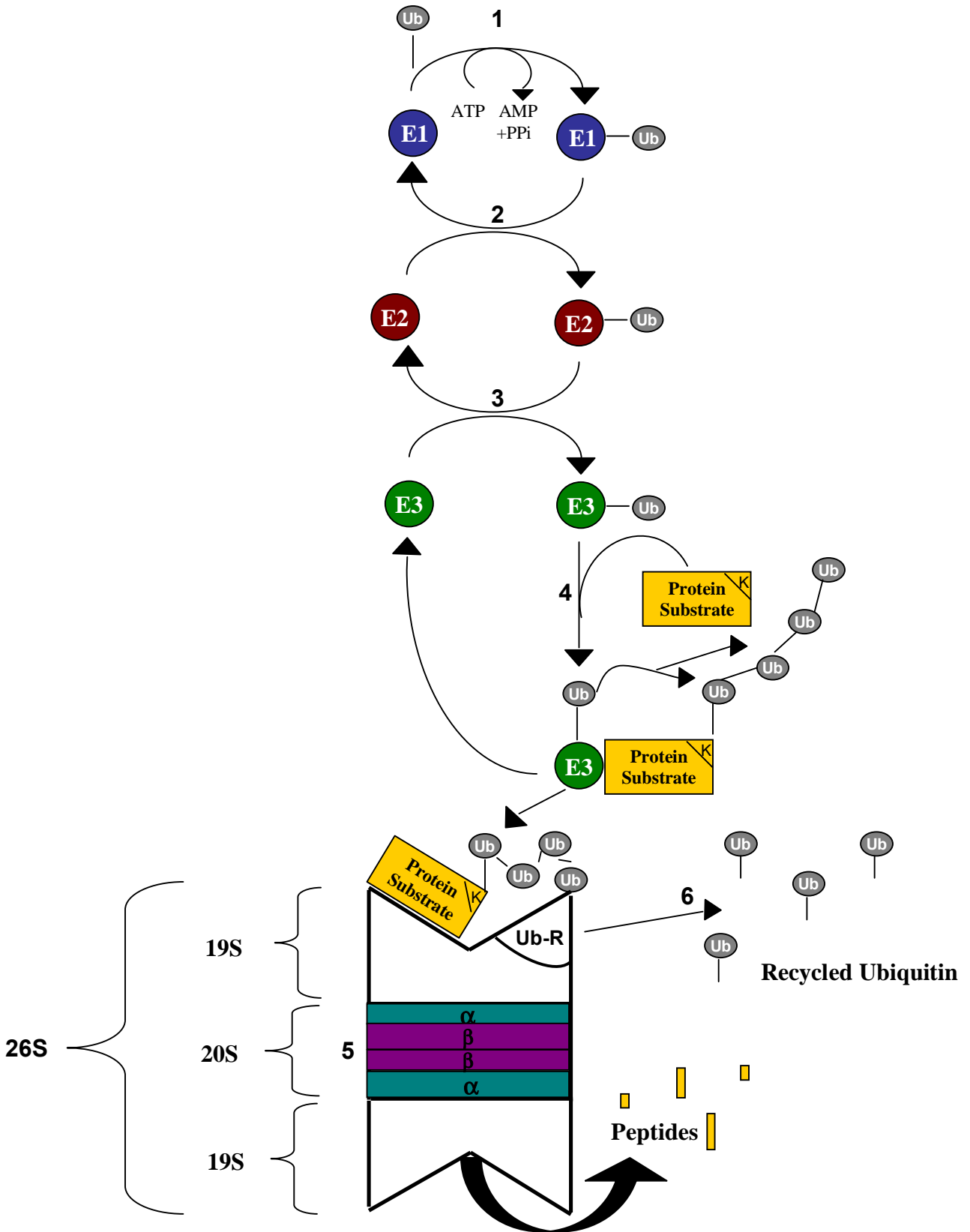
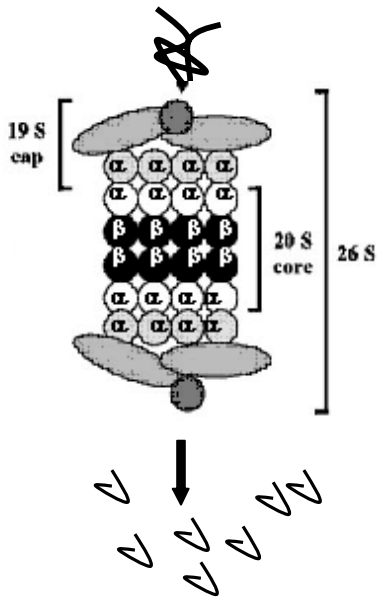


Figure 1-2. Model for the Age-Dependent Decrease in Ubiquitin-Proteasome System Activity in HD

A. Young Condition



B. Aged Condition

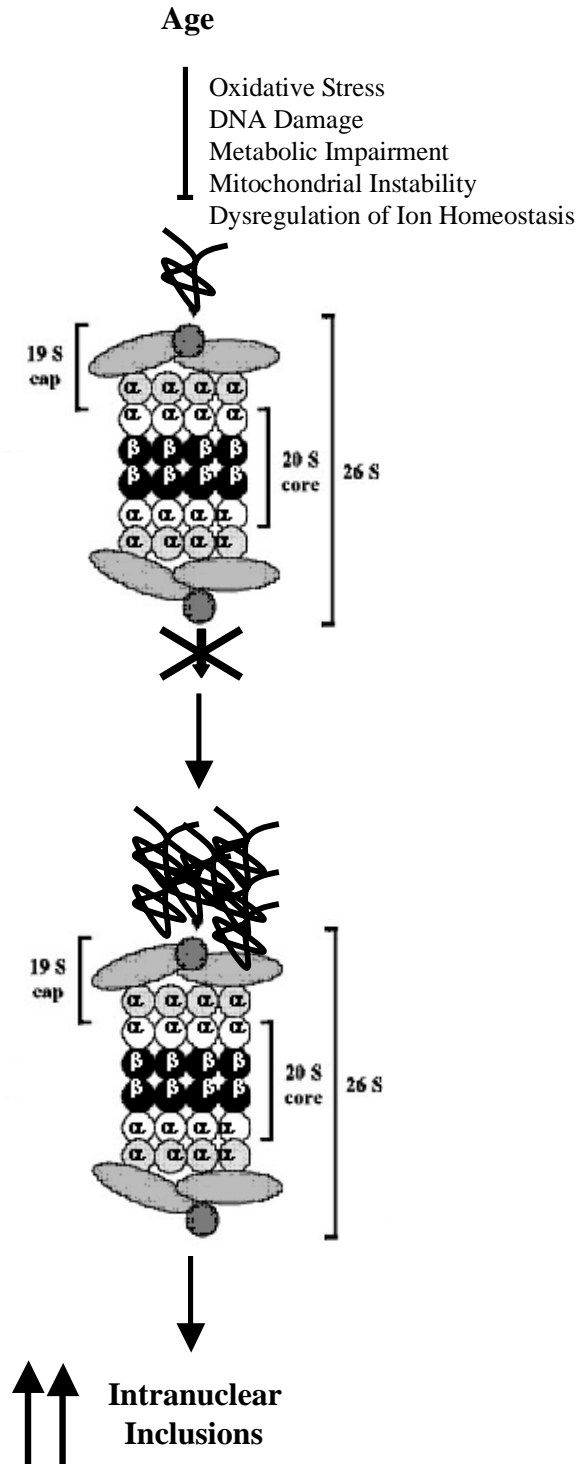


Table 1-2. Assays Used to Study Ubiquitin-Proteasome System Function

| Method | Measures | UPS component assayed | Advantages | Disadvantages |
|---|--|--|---|---|
| Fluorogenic substrate Peptides | 20S proteasome activity | Direct measure of chymotrypsin-like, trypsin-like or peptidyl-glutamyl proteasome activity | Quantitative analysis of proteolytic activity in cell and tissue lysates | Does not measure ubiquitylation, substrate interaction, unfolding or effects on other components of the UPS other than proteasome activity (Ding et al., 2002; Diaz-Hernandez et al., 2006) |
| Degron-tagged fluorescent proteins | Levels of fluorescent reporter protein tagged with a CL-1 degron targeting it for proteasome degradation | Proteasome activity and targeting to proteasome | Functional analysis of UPS system <i>in vivo</i> of a single cell or group of cells | Does not measure all aspects of UPS function (Bence et al., 2001) |
| Ub ^{G76V} -GFP transgenic Mice | Levels of fluorescent reporter protein tagged with G76V substitution to efficiently ubiquinate it for proteasome degradation | Proteasome activity and targeting to proteasome in a live mouse | Functional analysis of UPS system in a live mouse | Does not measure all aspects of UPS and has very low expression in the brain (Lindsten et al., 2003) |
| Yeast two-hybrid assay | Interaction of polyglutamine containing proteins with UPS components | Proteasome subunits and components that interact with polyQ Proteins | Shows direct interaction | Does not give functional data (Davidson et al., 2000) |
| In vitro assay of proteasome activity | Effect of synthetic peptides, purified aggregates and fibrillar species on activity of purified proteasome | Activity of purified proteasomes | Shows direct effects of polyQ containing proteins on proteasome activity | Does not measure other components of the UPS (Bennett et al., 2005; Ventkatraman et al., 2004) |

Figure 1-3. Hypothesized Results

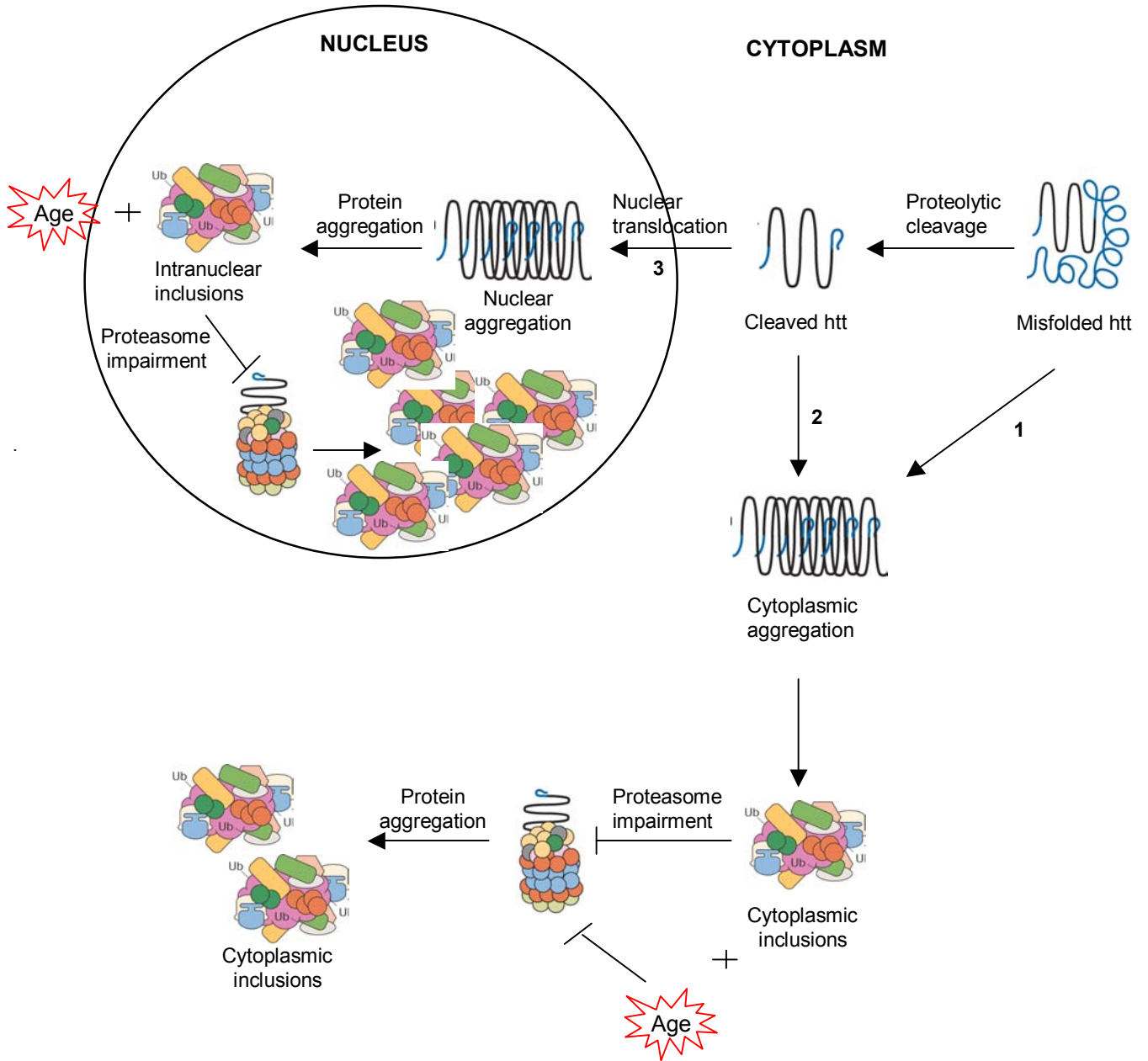


Figure 2-1. Model for the N-End Rule Pathway that Governs the Proteasome Reporter GFPu

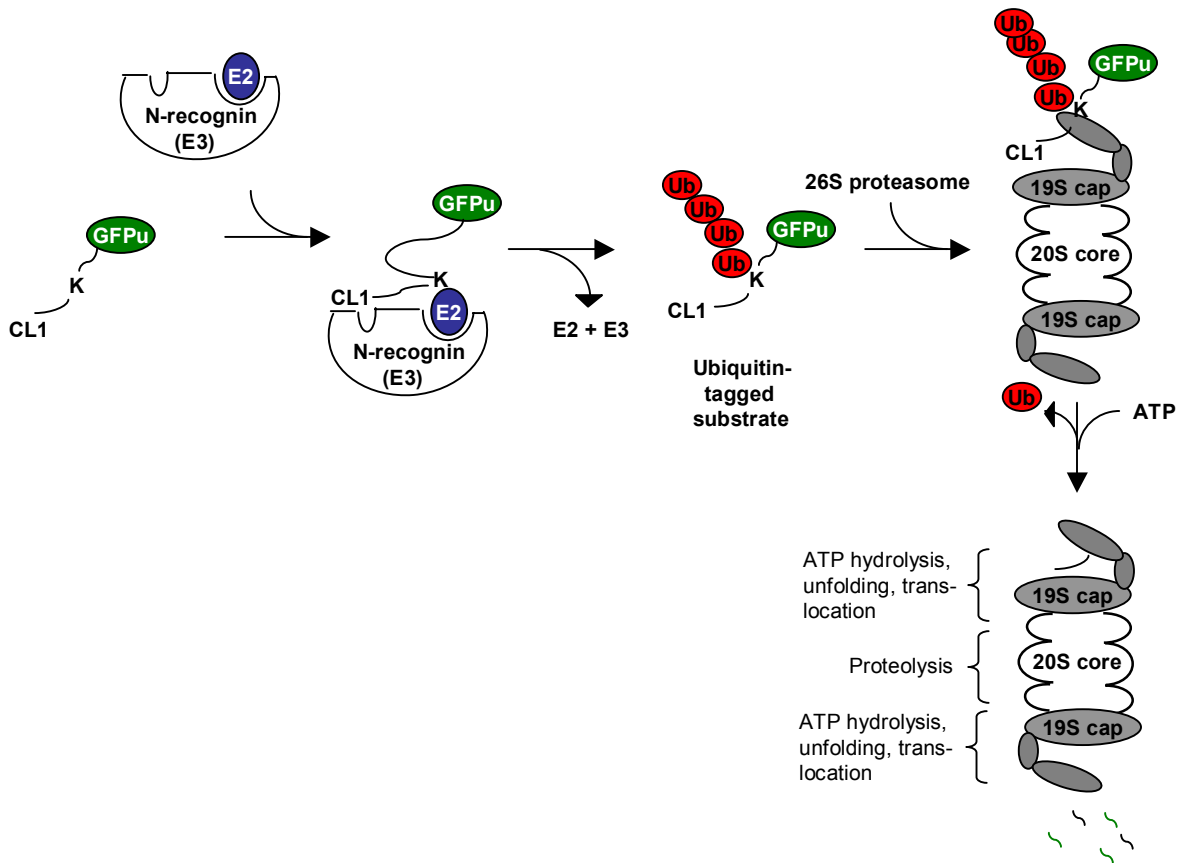


Fig.2-2

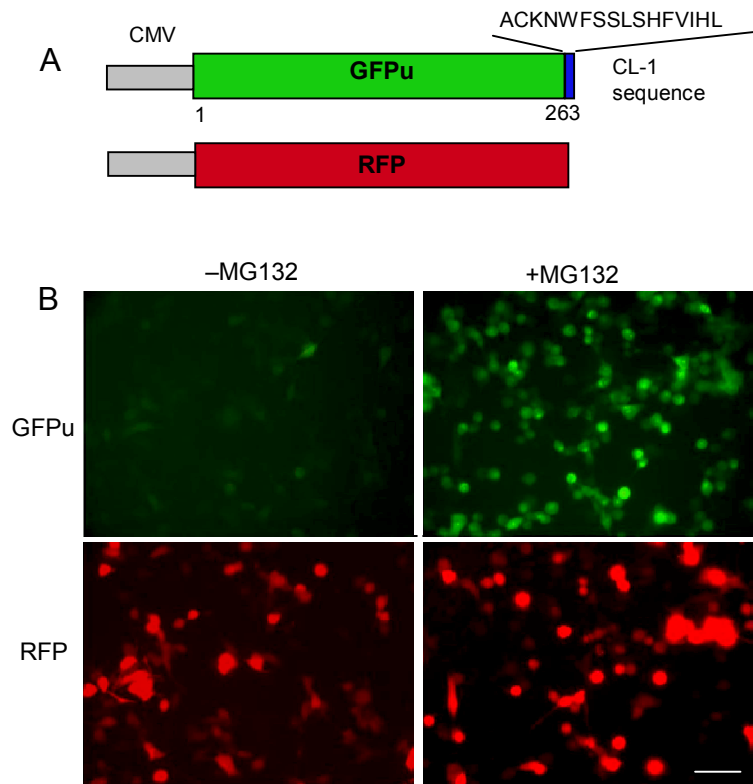


Fig. 2-3

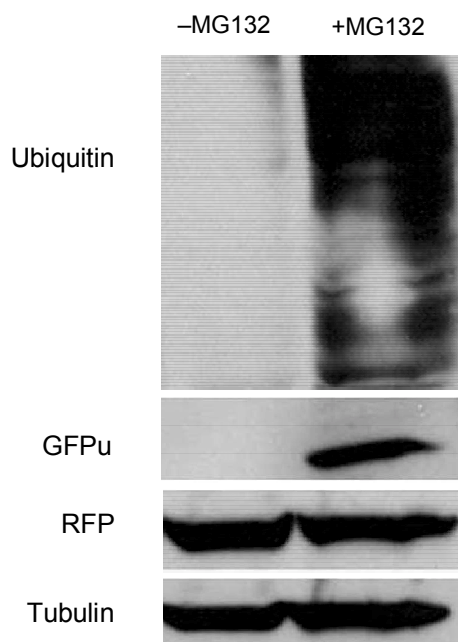


Fig. 2-4

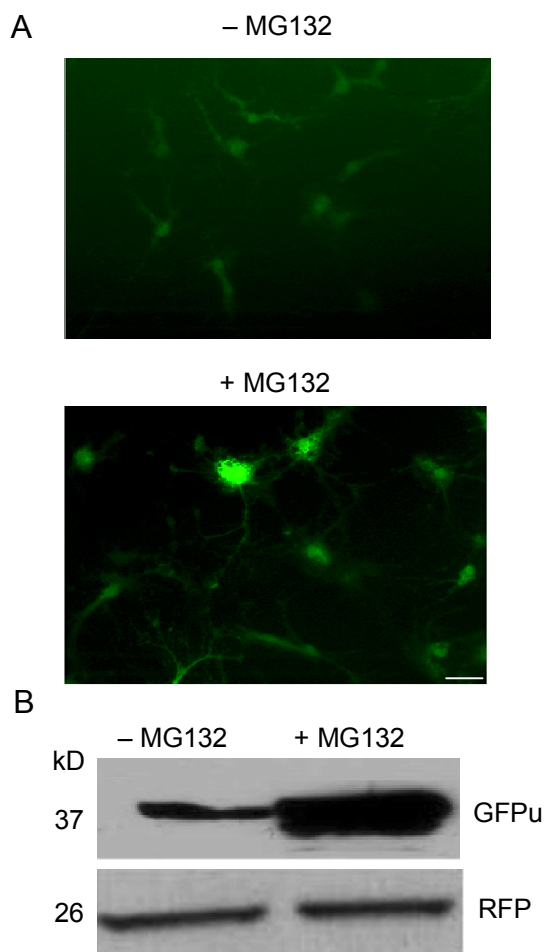


Fig. 2-5

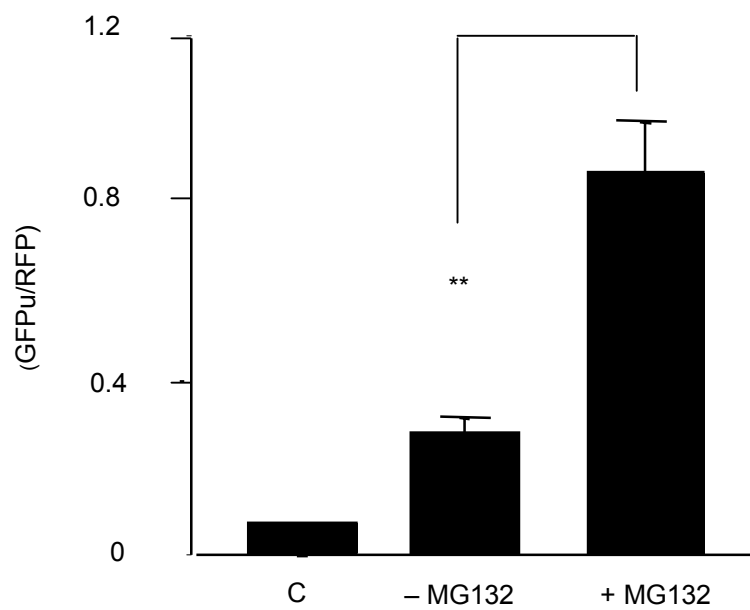
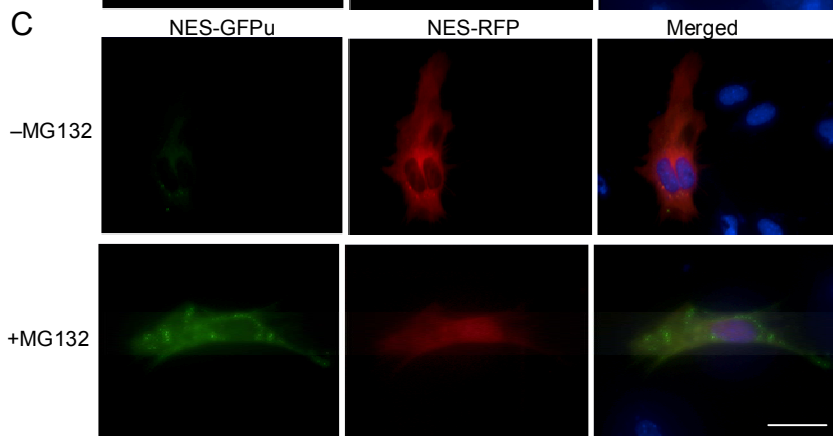
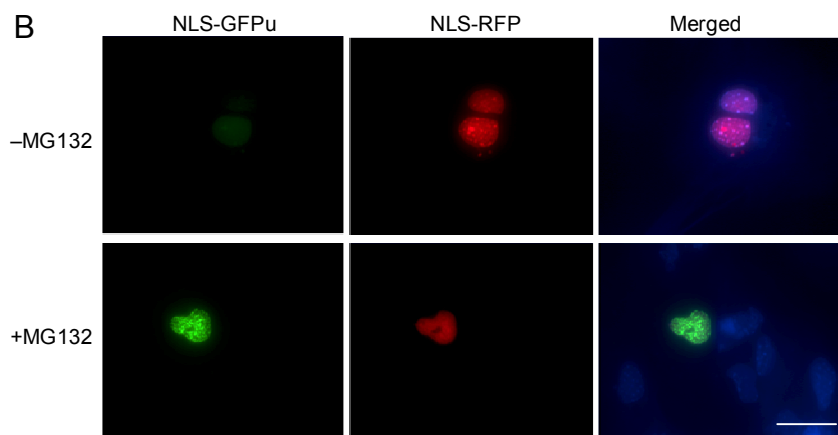
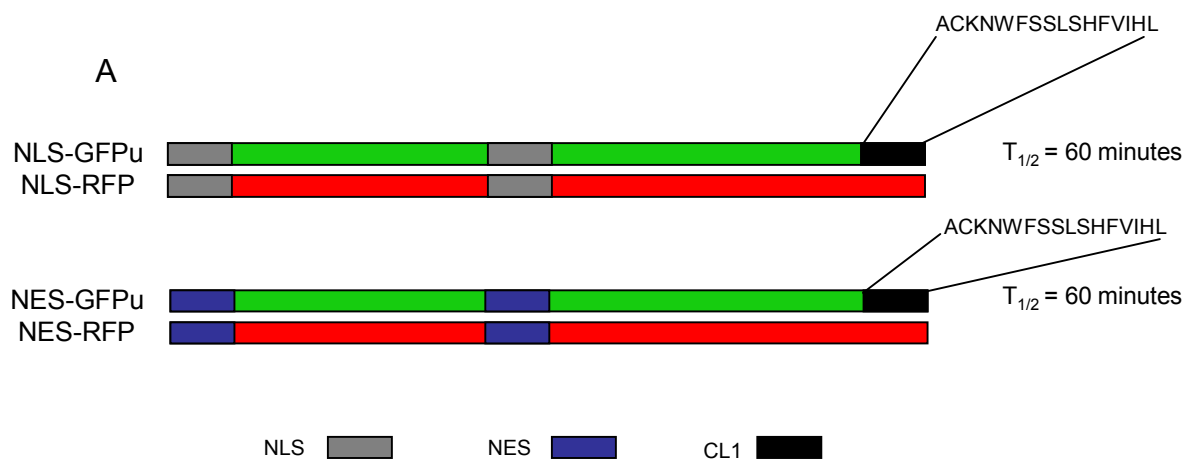


Fig. 2-6



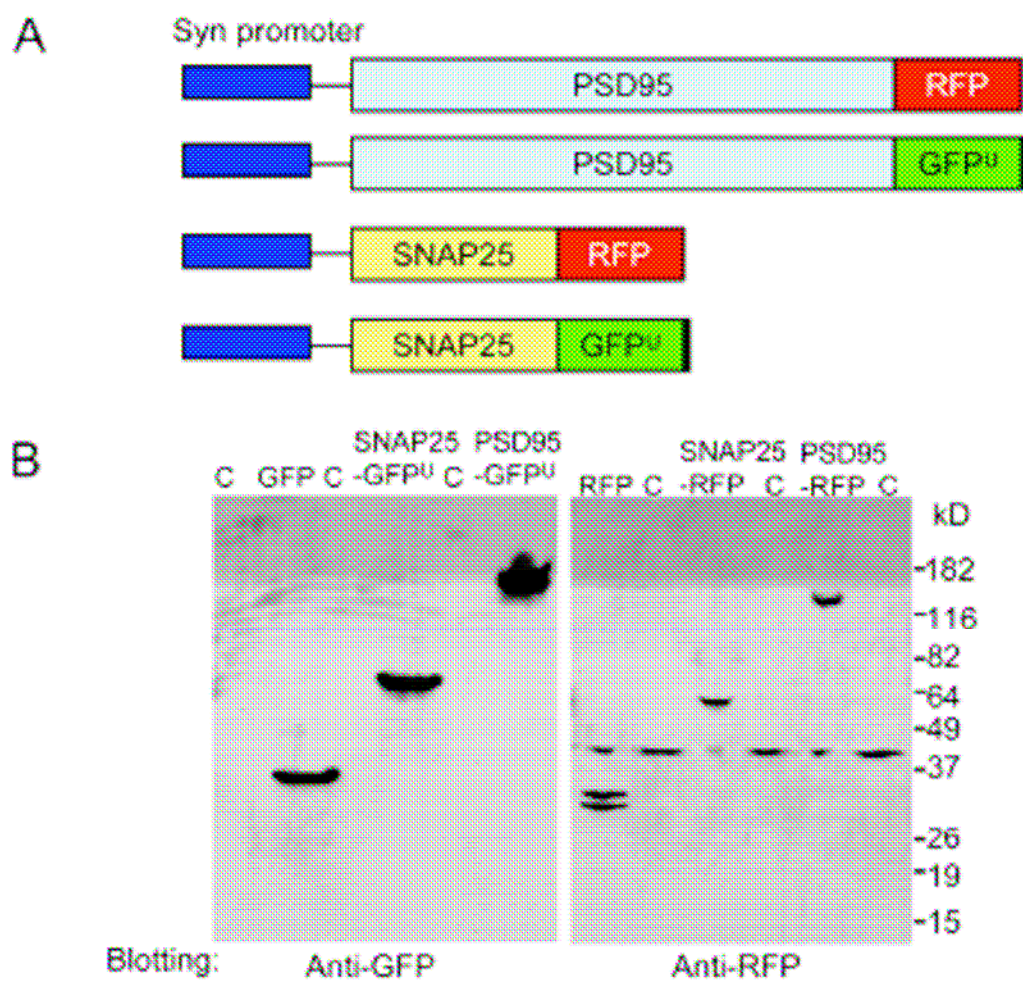


Fig. 2-8

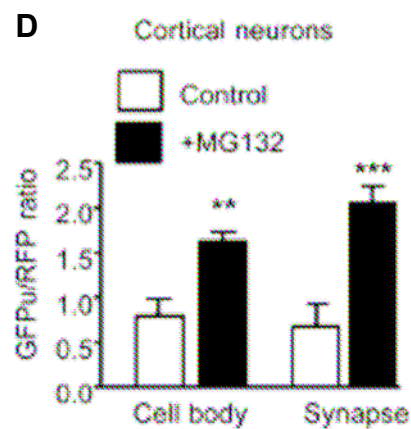
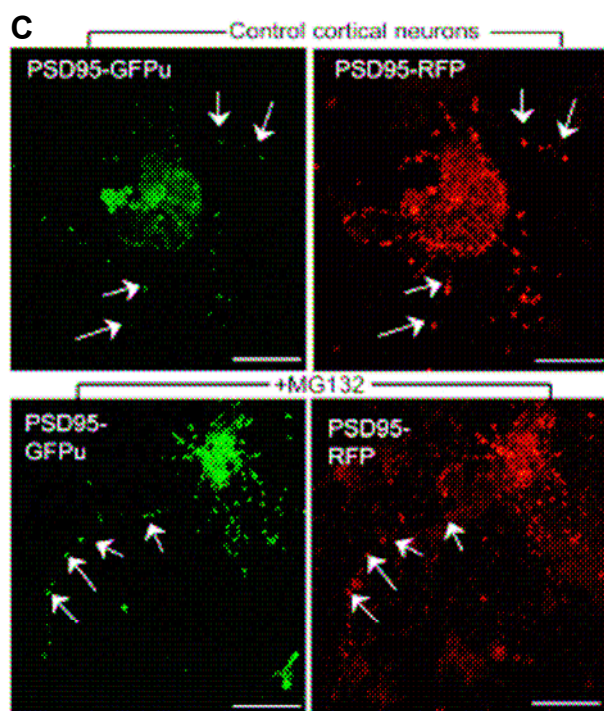
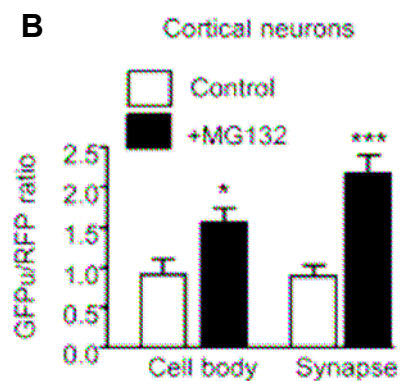
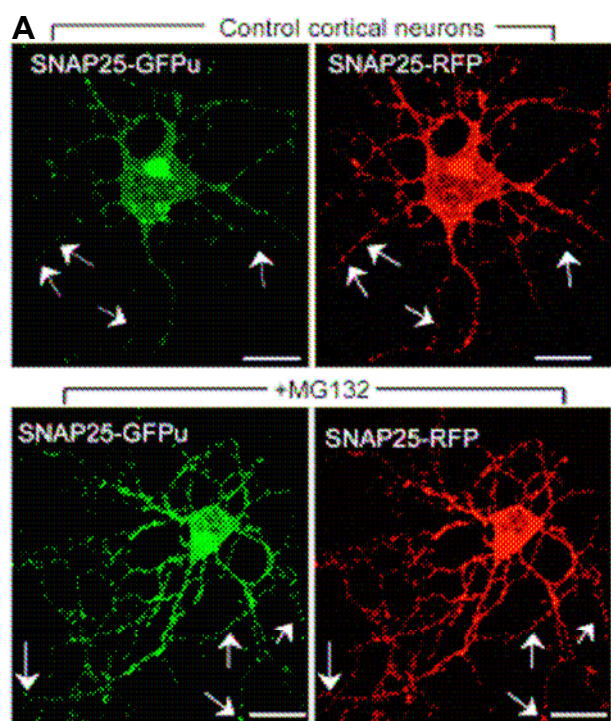


Fig. 3-1

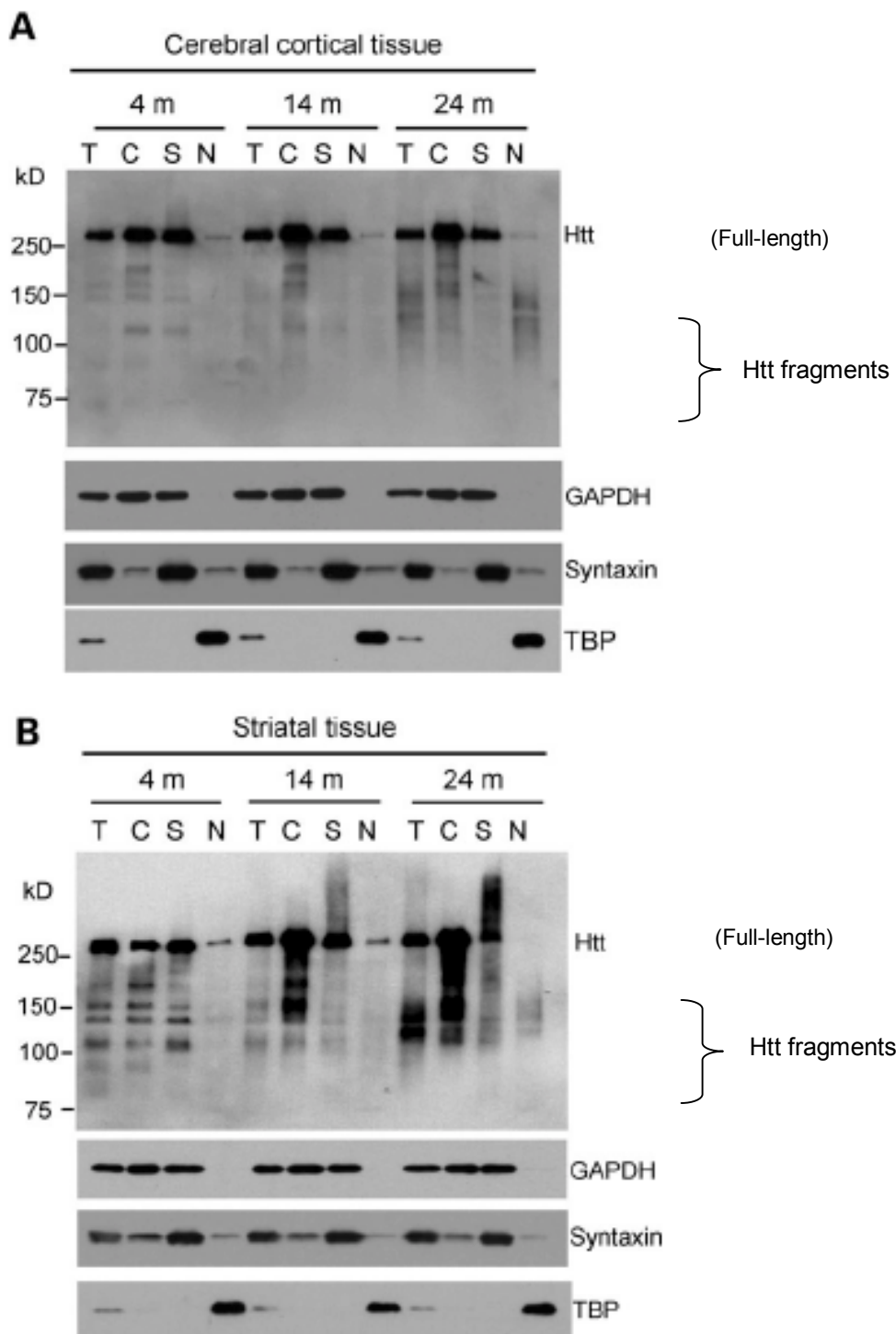


Fig. 3-2

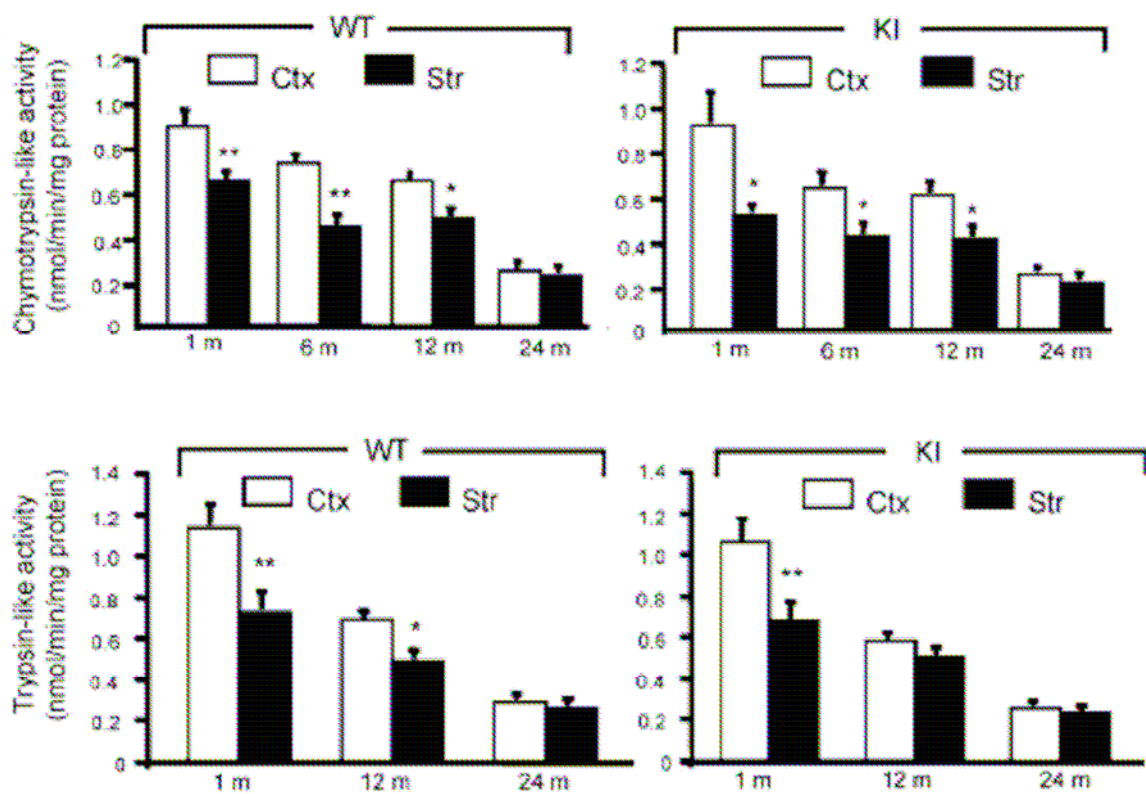
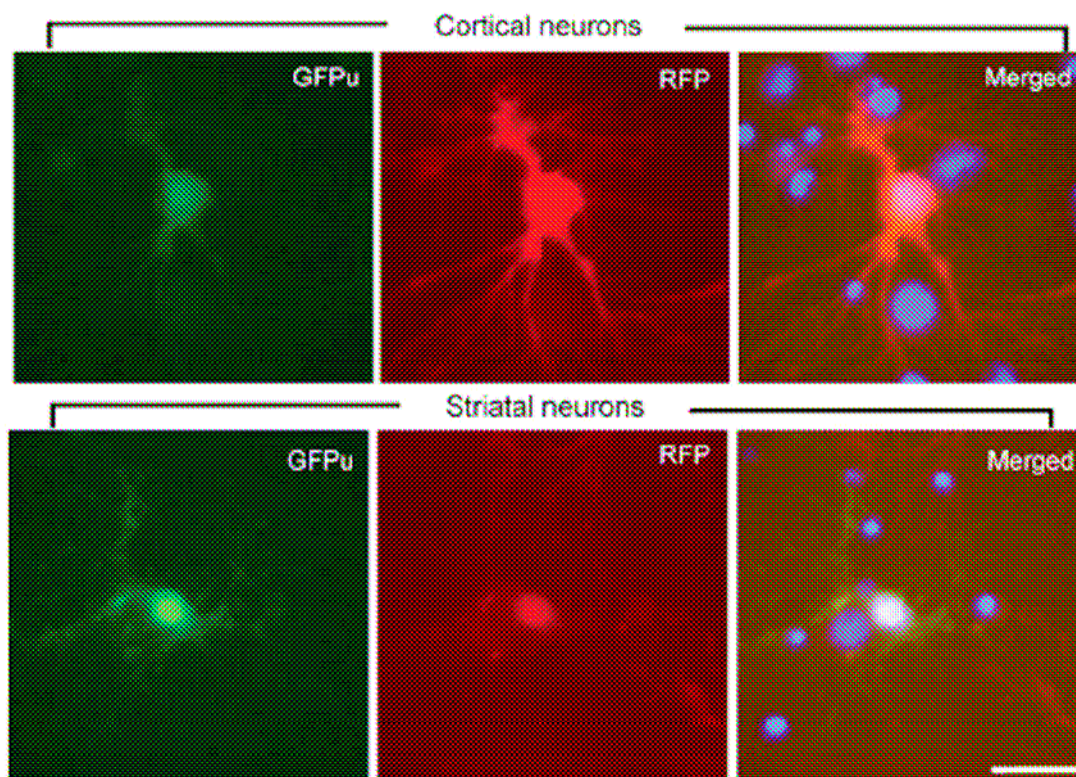


Fig. 3-3

A.



B.

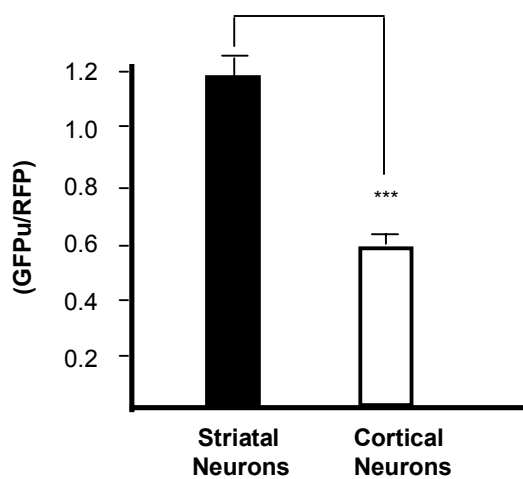


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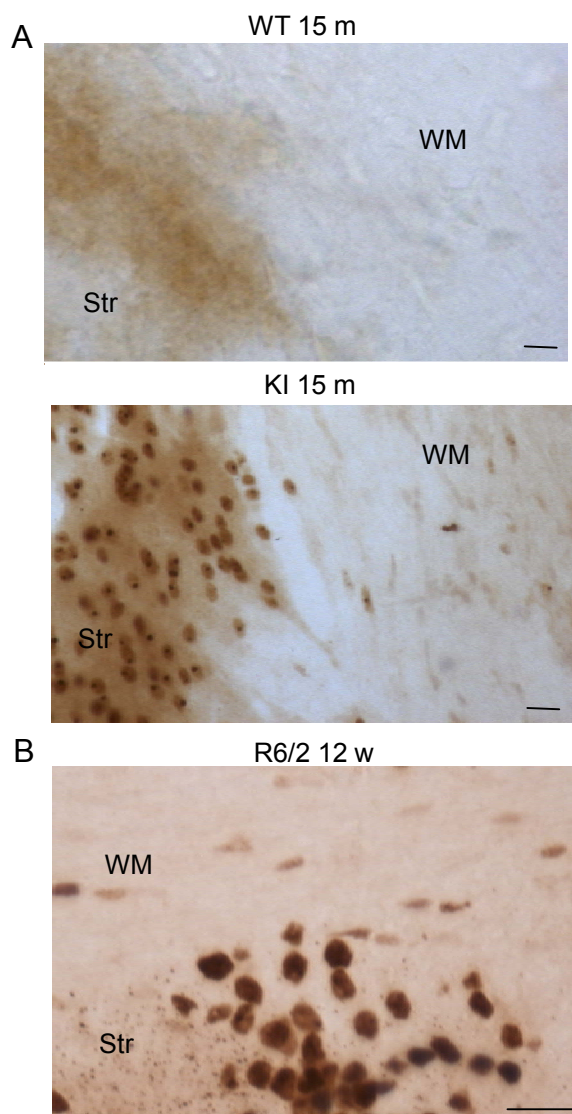


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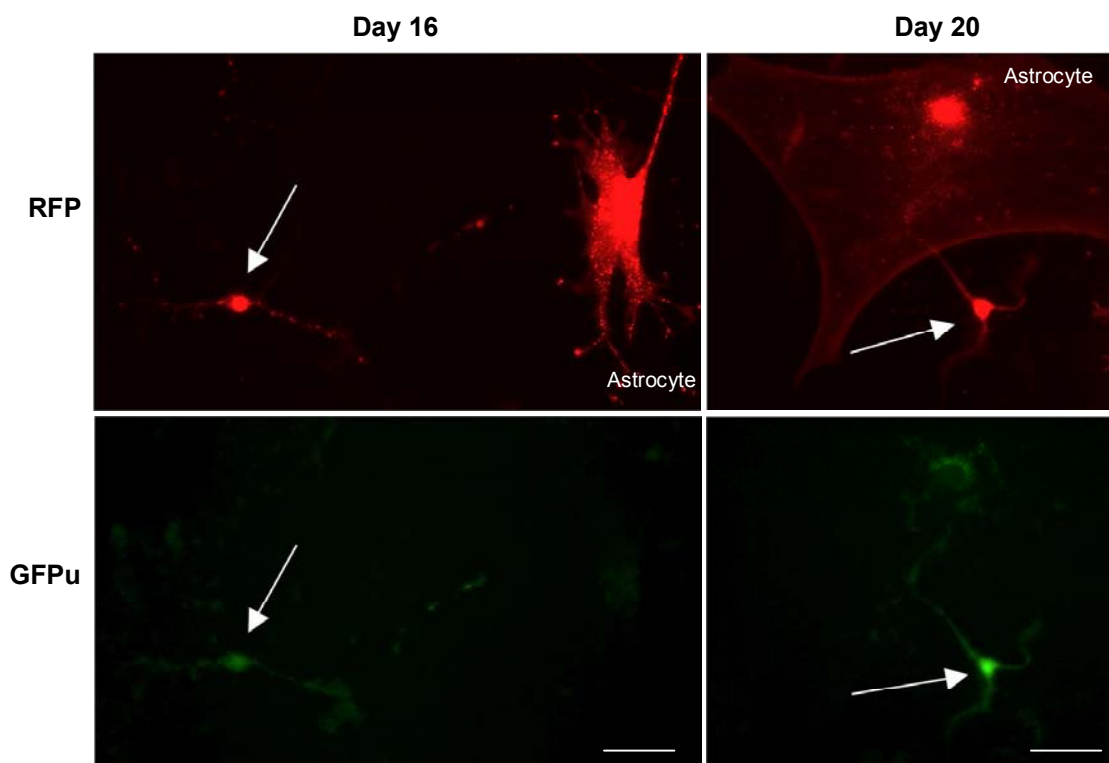


Fig. 3-6

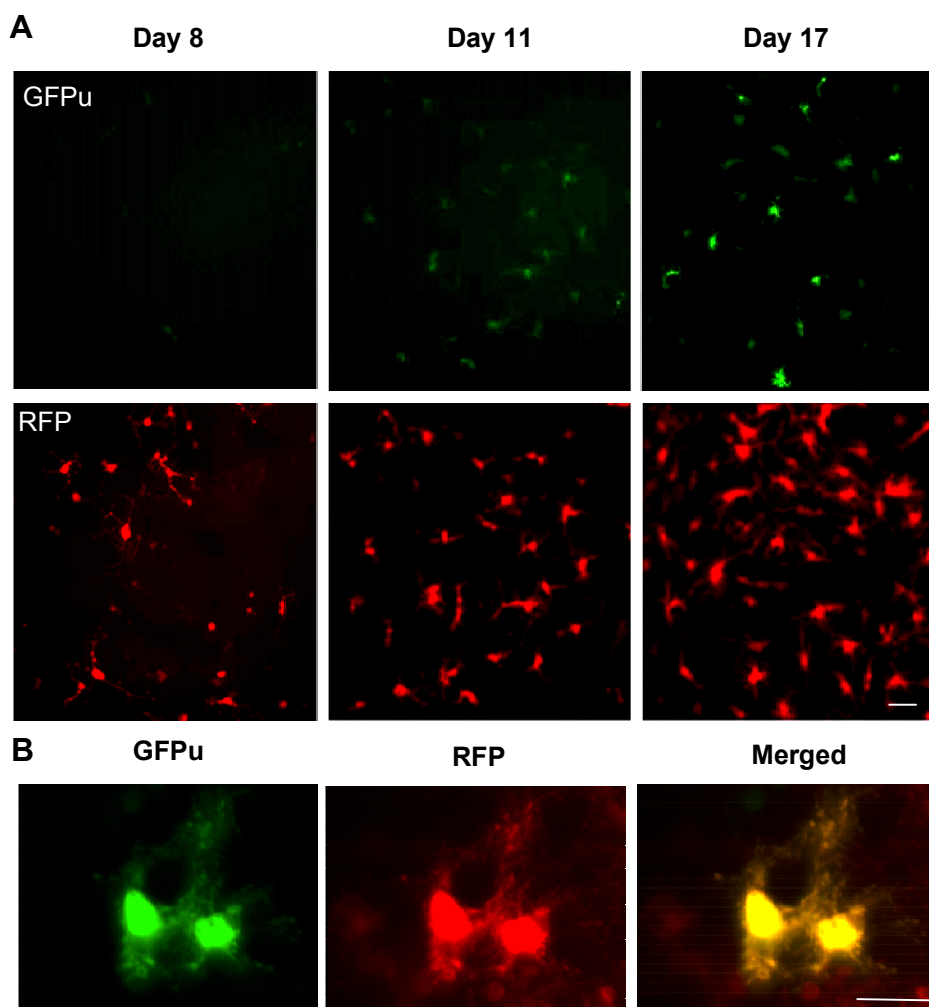


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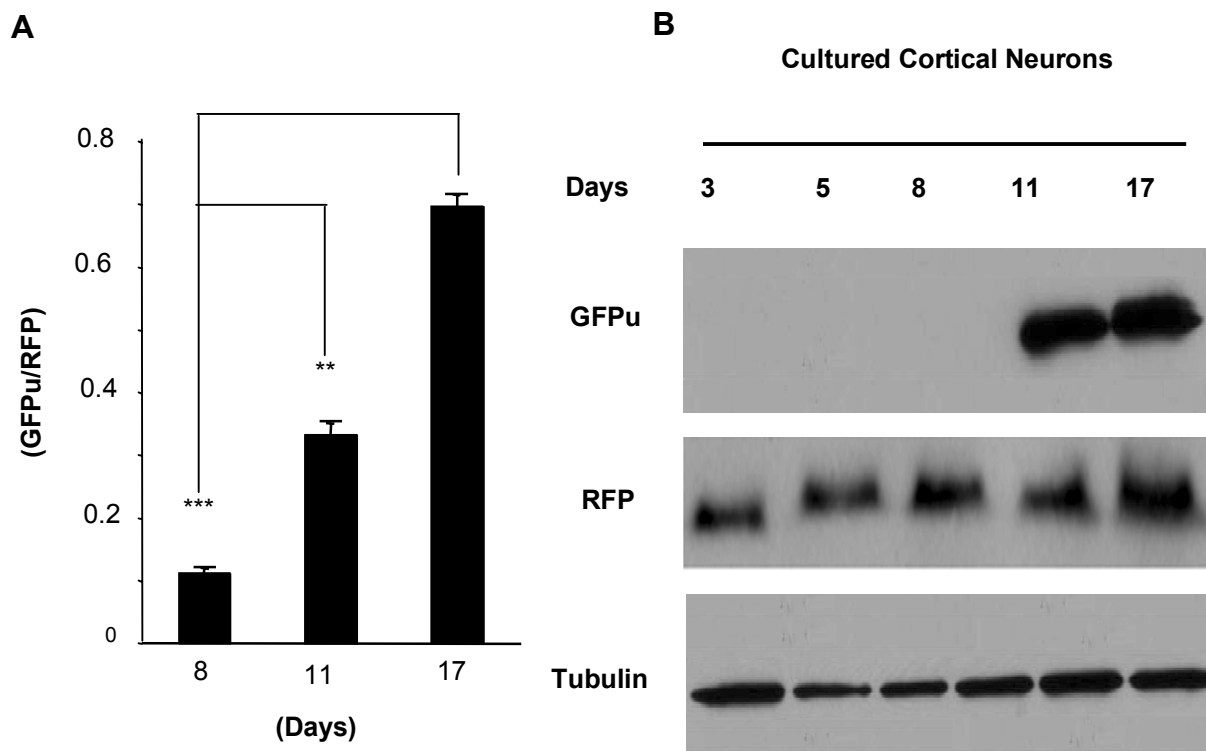


Fig. 3-8

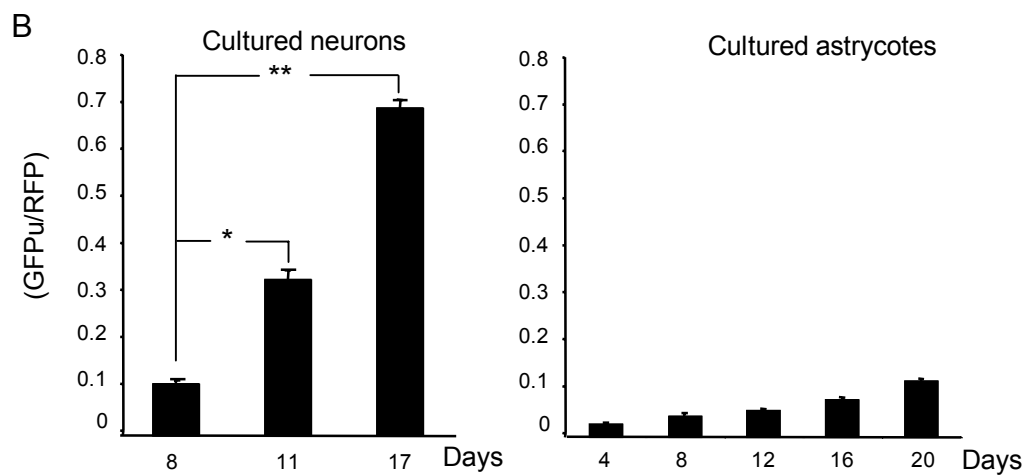
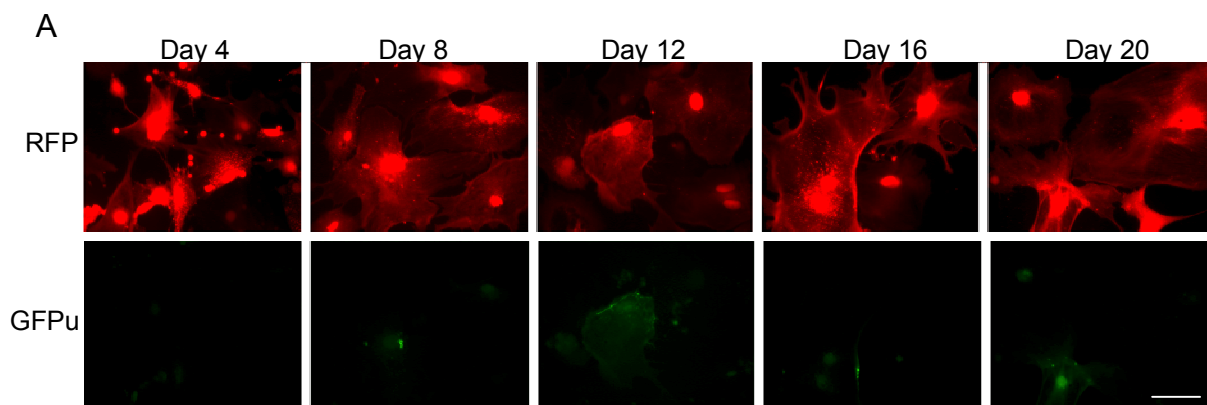


Fig. 3-9

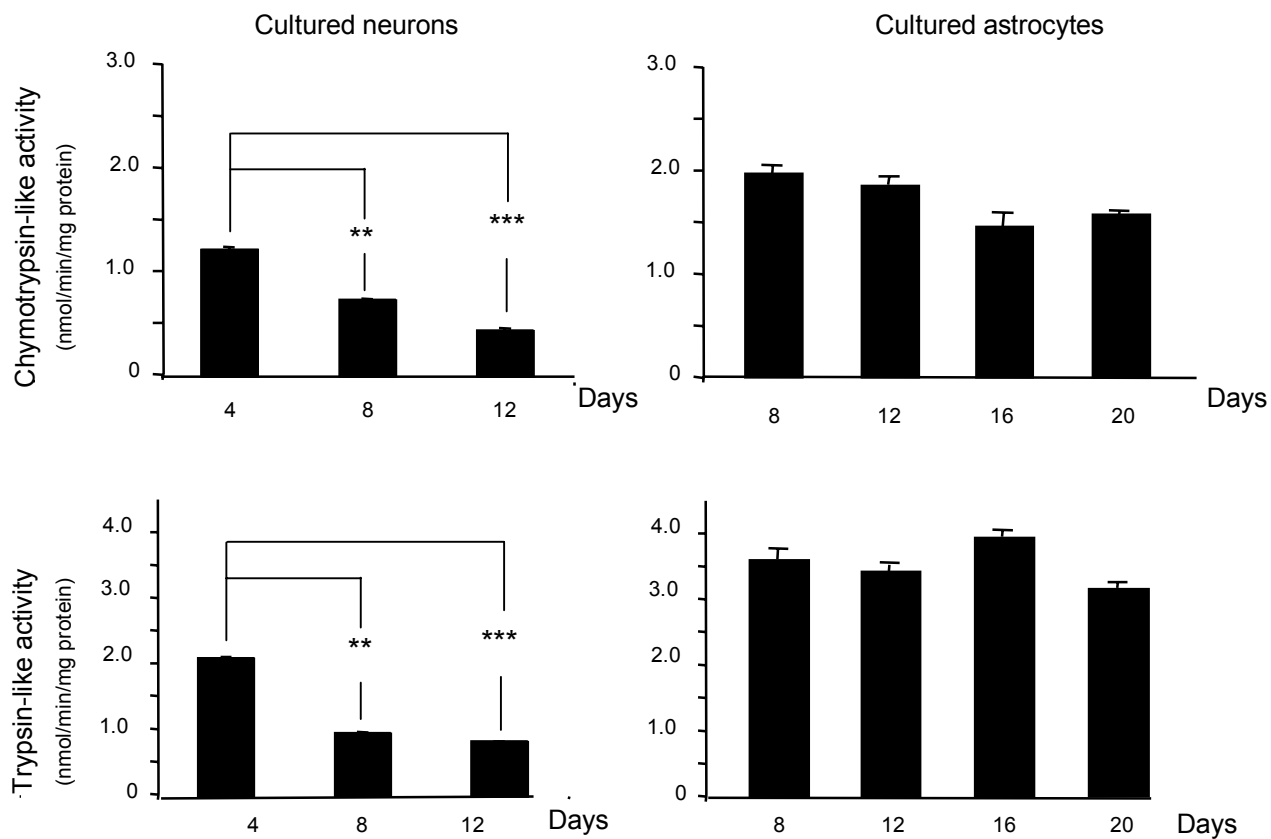


Fig. 3-10

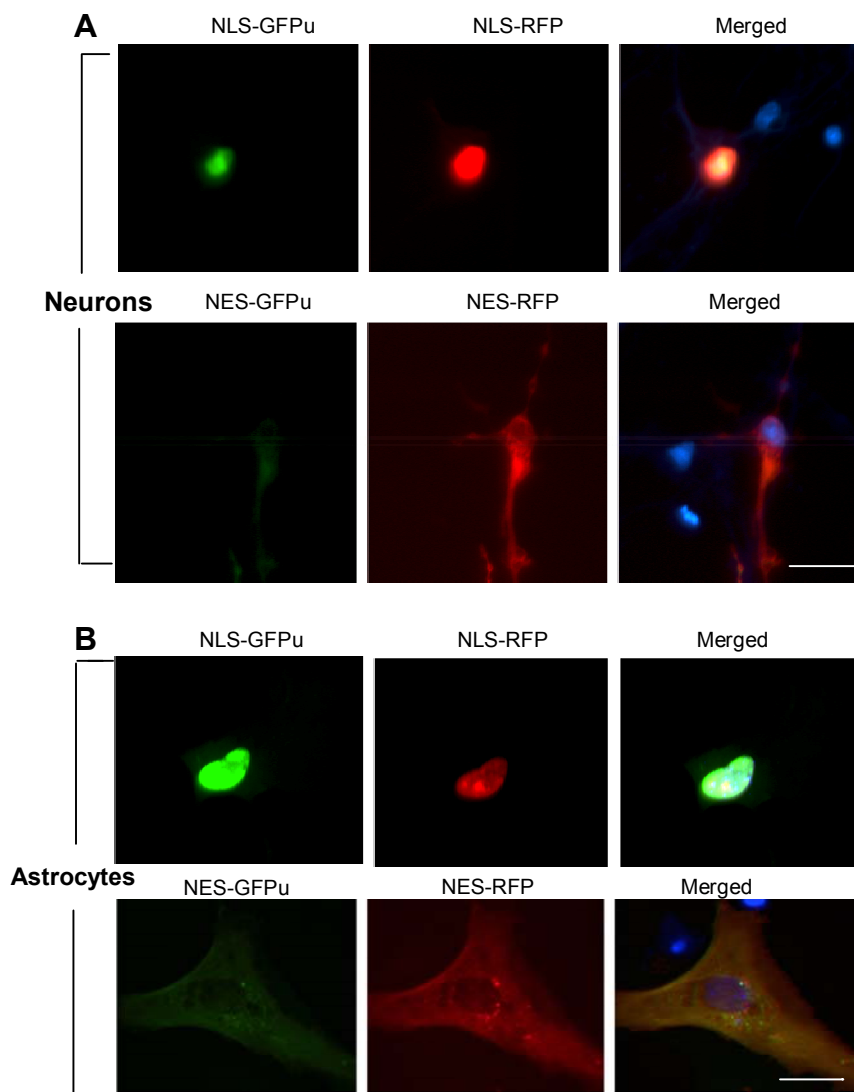


Fig. 3-11

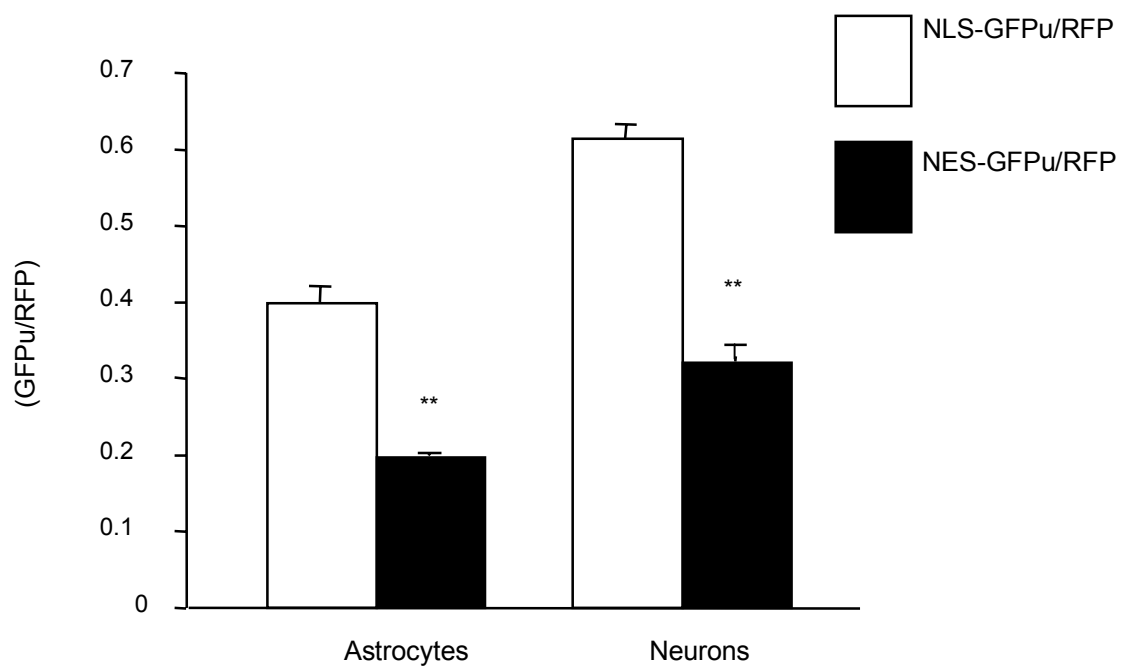


Fig. 4-1

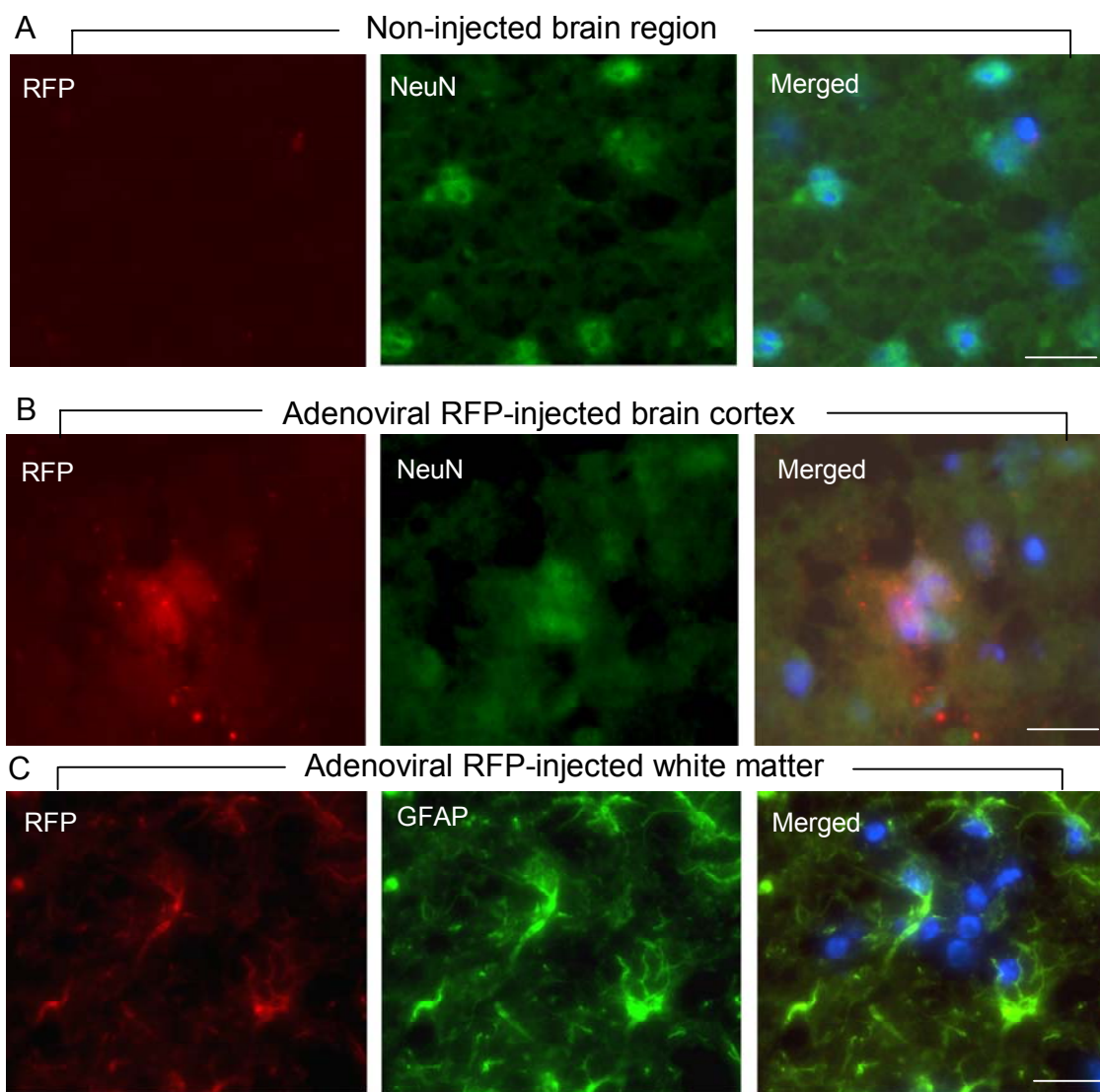


Fig. 4-2

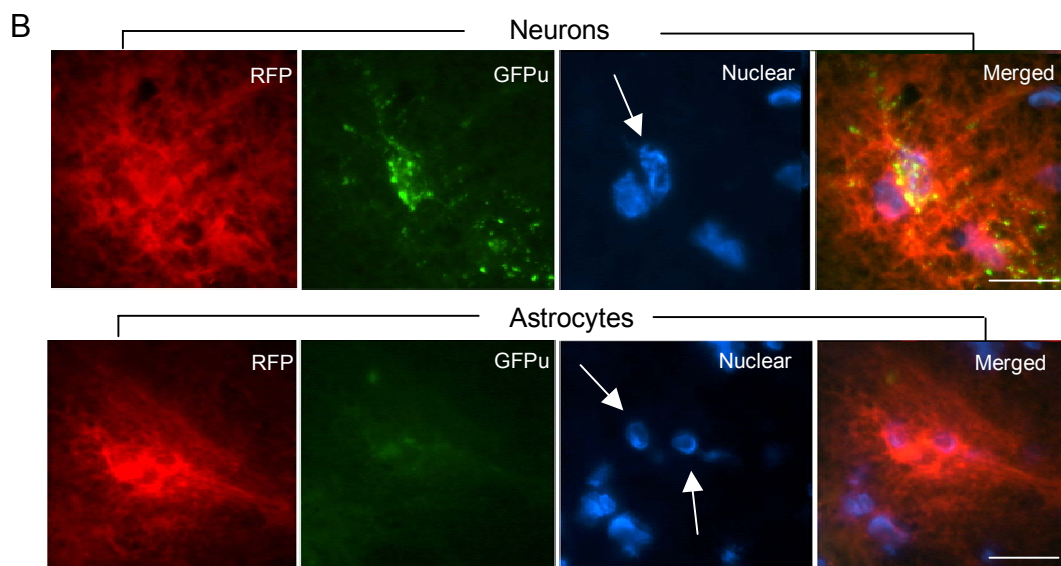
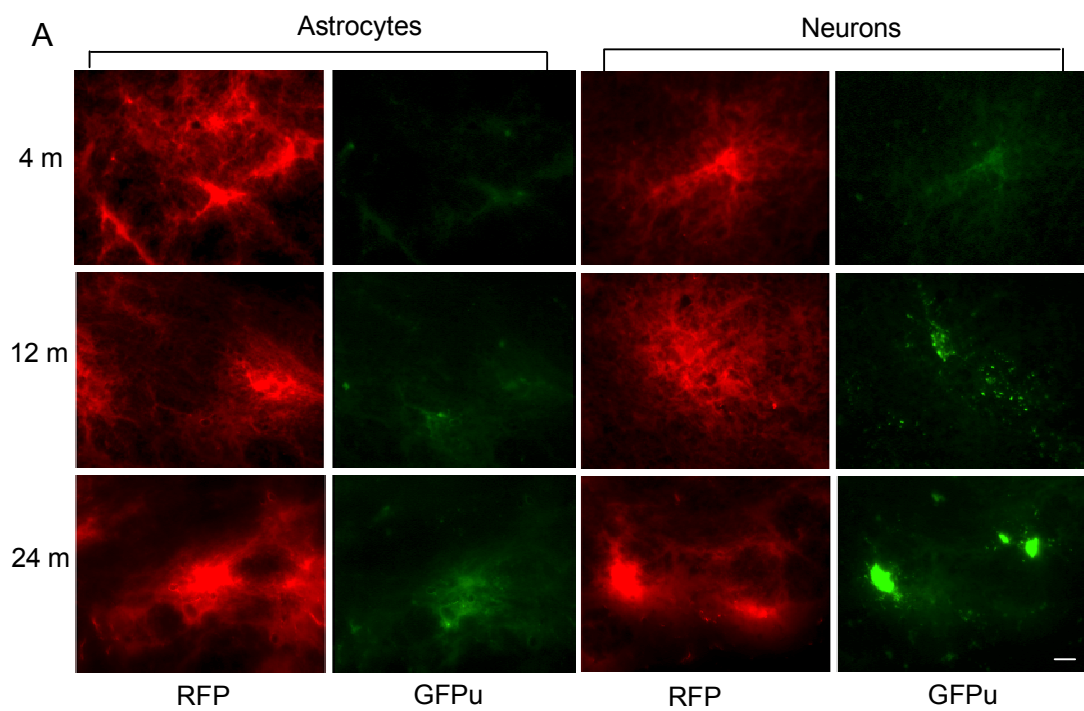


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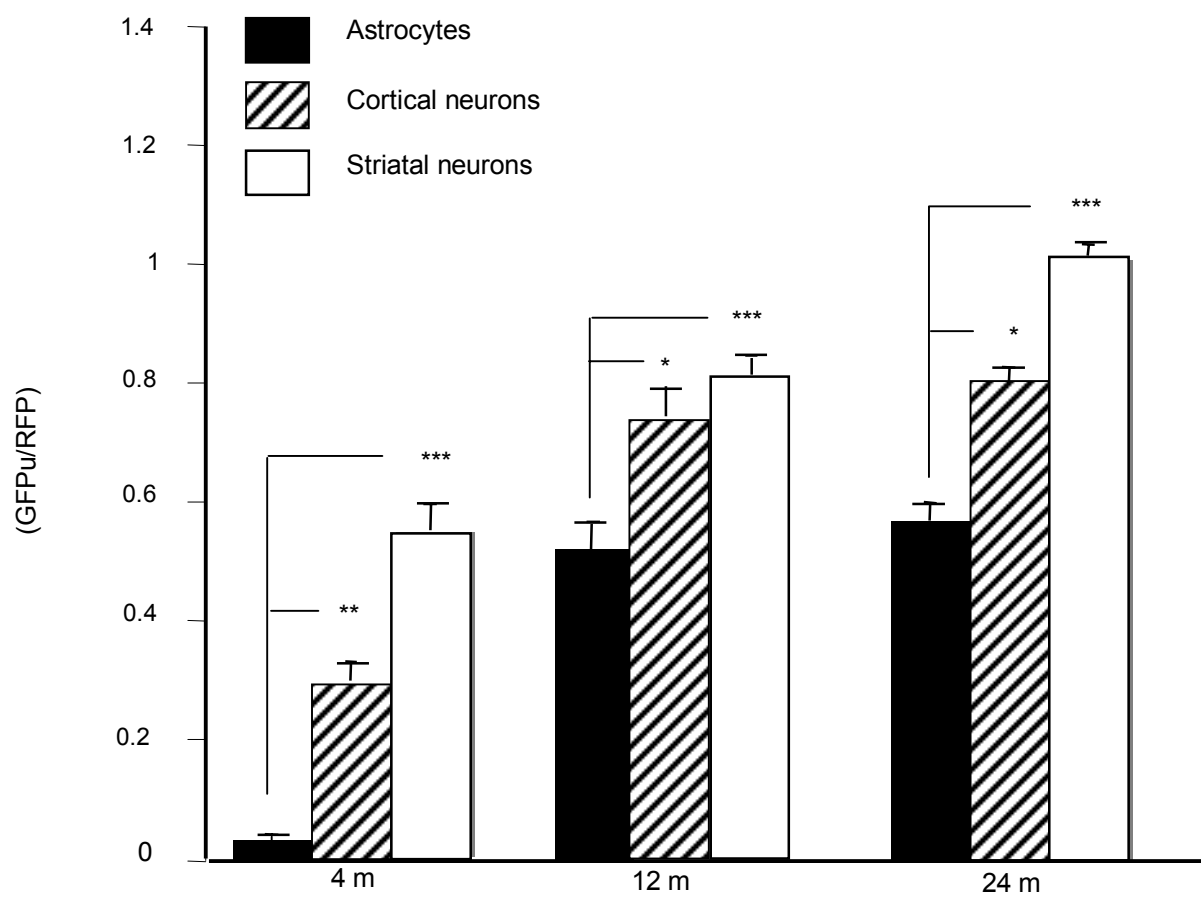


Fig. 4-4

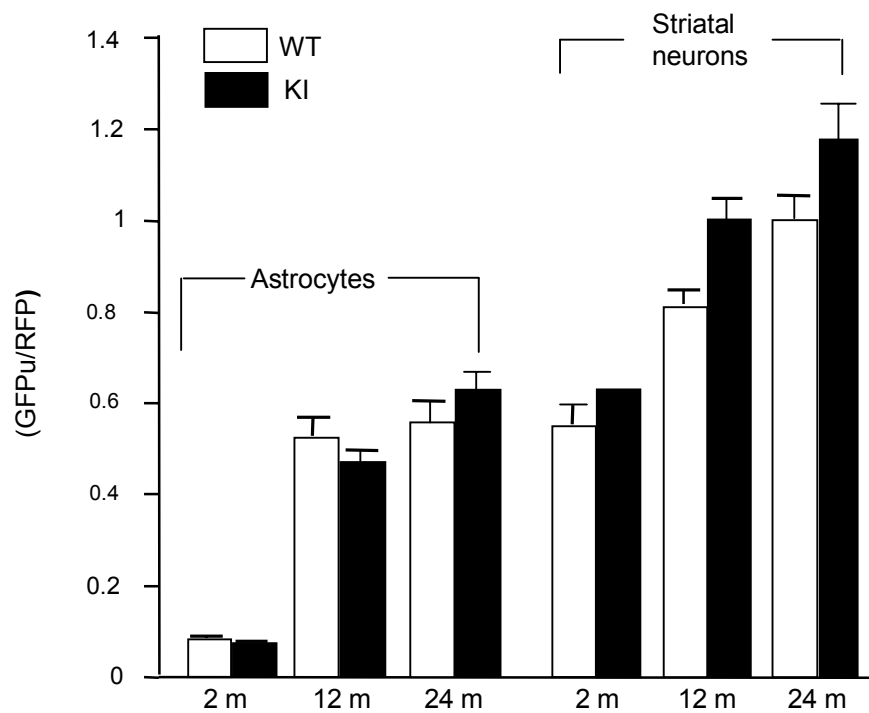


Fig. 4-5

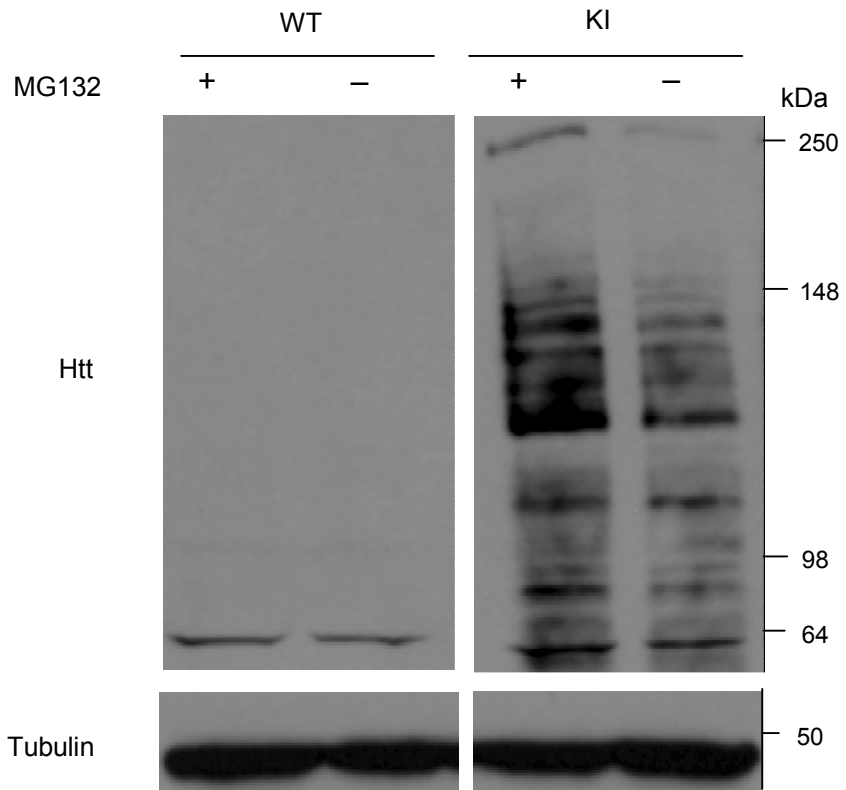


Fig. 5-1

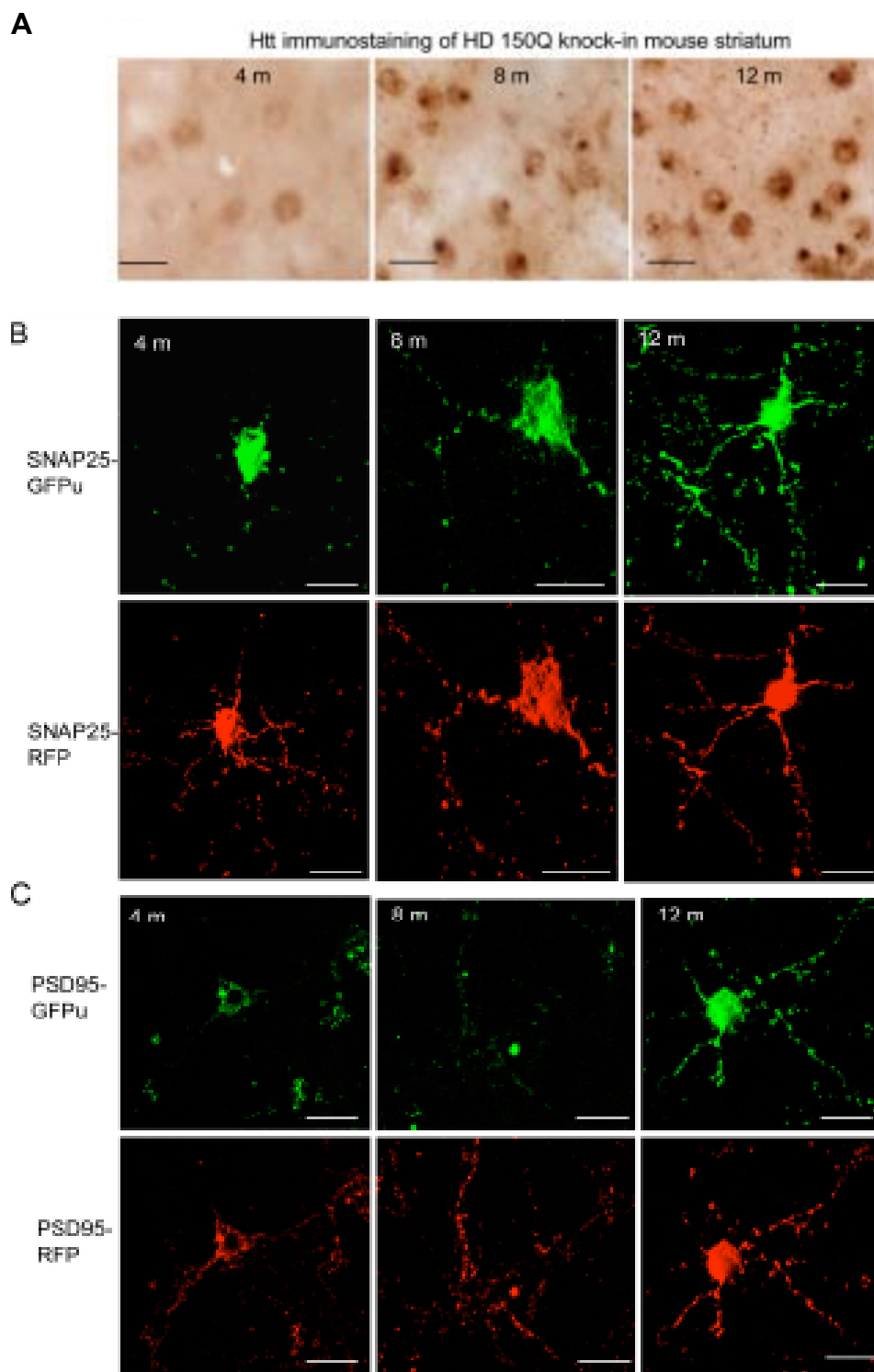


Fig. 5-2

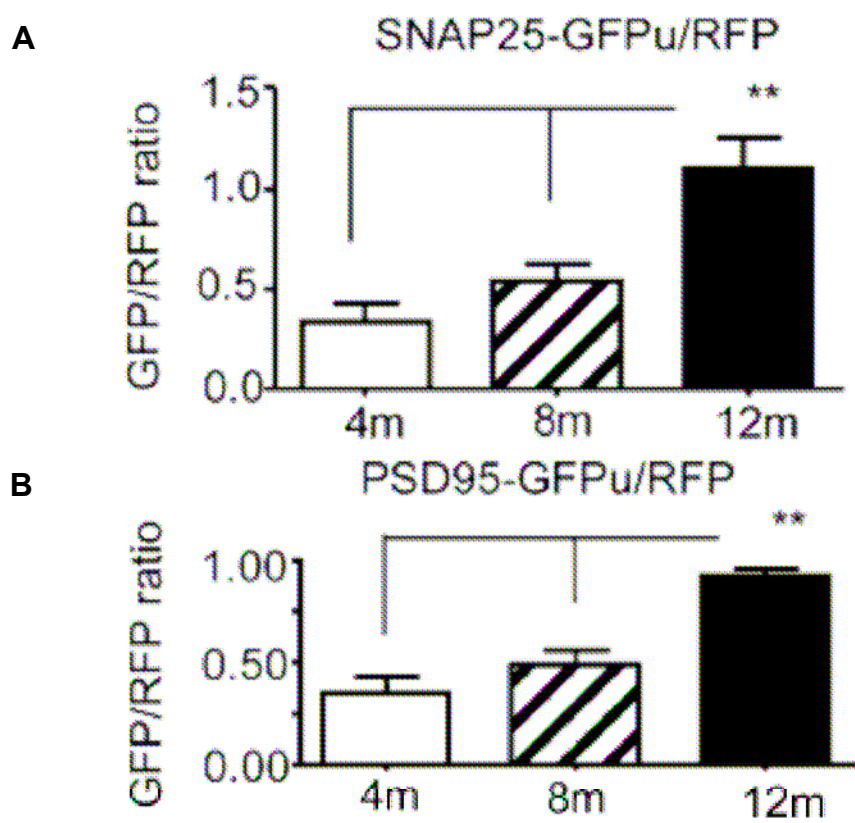


Fig. 5-3

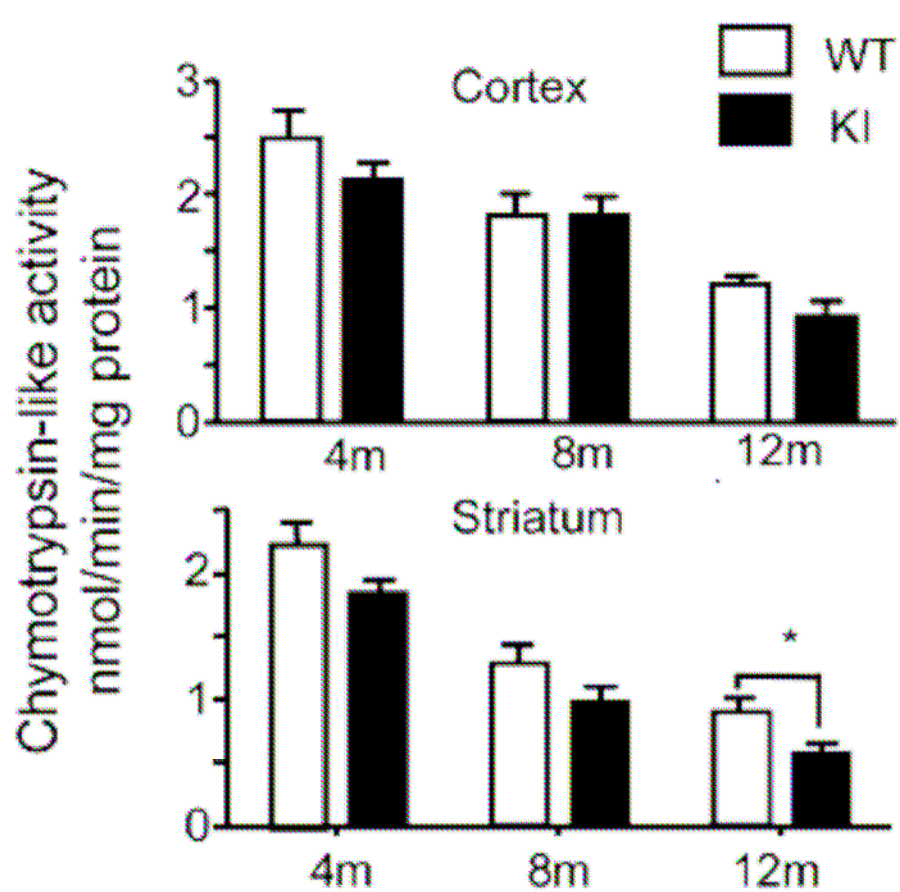
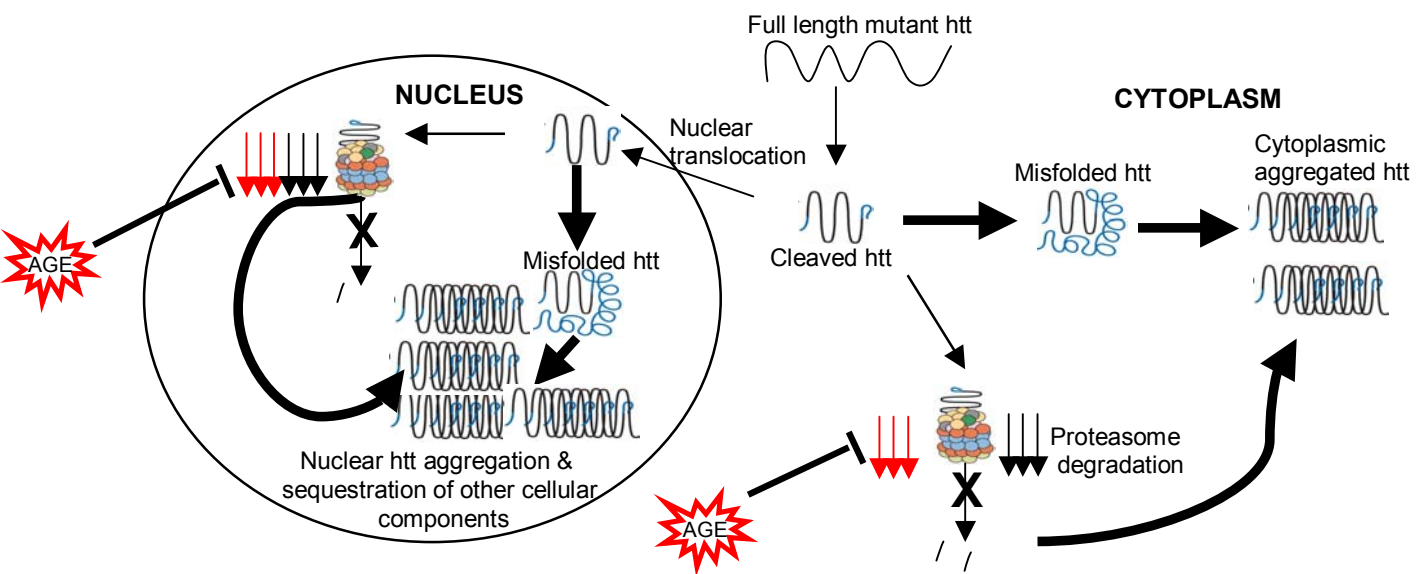


Fig. 6-1

A. Neuron



B. Astrocyte or other cell type

