

## Supplementary Materials

### Supplementary Figure 1 TG6 is expressed in cortical neurons.

Immunocytochemical analysis of TG6 and Map2 in DIV7 primary cortical neurons showed that TG6 is mostly expressed in the nuclear compartment (co-localization with DAPI) and only marginally with the ER (co-localization with Calnexin). Bar, 10 micron. Shown are representative images of three cerebellar cultures. TG6, calnexin and Map2 were detected with specific antibodies, nuclei were detected with DAPI.

### Supplementary Figure 2 HA and V5 tags do not affect expression and activity of TG6.

C-terminal HA (A) and V5 (B) tags do not affect TG6 expression or activity compared to untagged TG6. Graph, mean  $\pm$  SEM, n = 3, ns=not significant; \*p<0.05; \*\*\*p<0.001, 1-way ANOVA with Tukey's *post hoc* test.

### Supplementary Figure 3 Mutations in TG6 do not alter TG6 transcript levels.

Real Time-PCR analysis of the transcript levels of TG6-WT, TG6-R111C and TG6-D327G expressed in HEK293T cells and normalized to actin. Graph, mean  $\pm$  SEM, n = 3. ns=not significant; 1-way ANOVA with Tukey's *post hoc* test.

### Supplementary Figure 4 Expression of TG6-WT, TG6-R111C and TG6-D327G in primary neurons reflects the subcellular localization and rate of protein degradation observed in HEK293T and COS-7 cells.

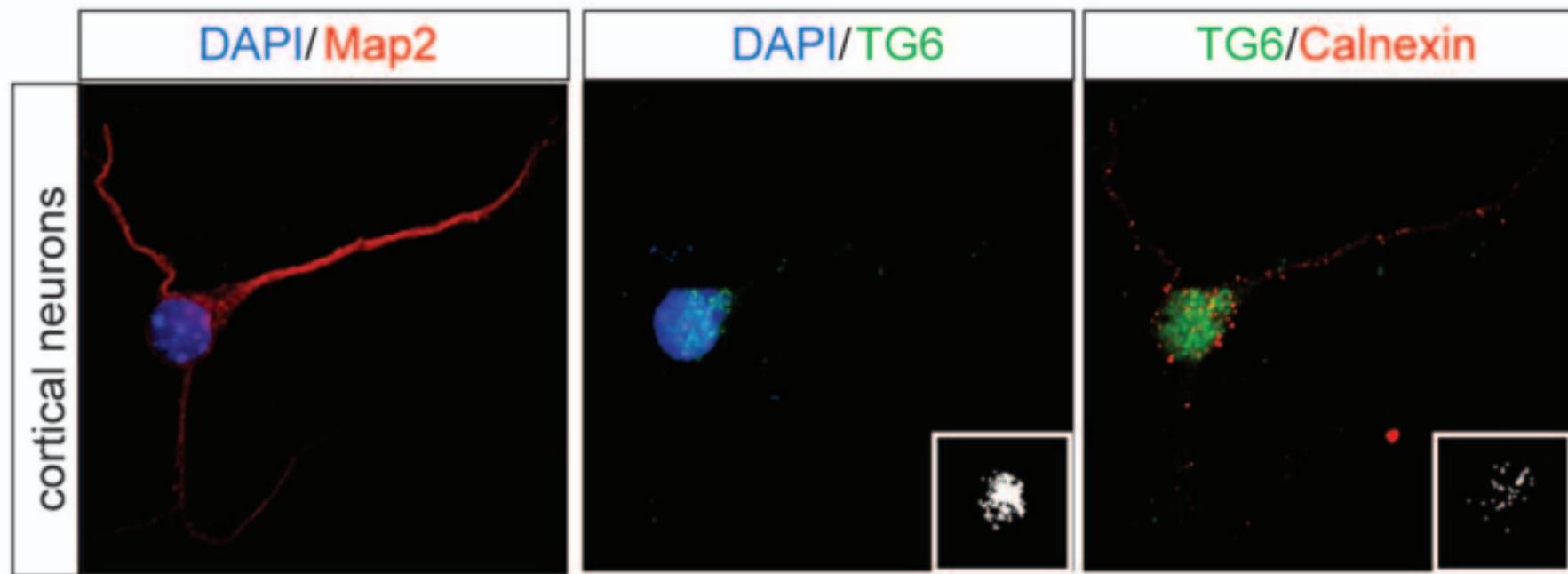
A) Representative images showing expression of the transgene in primary cortical neurons at DIV5 via viral transduction. Expression of both GFP and TG6-WT or TG6 mutants was driven by two independent synapsin promoters, thereby enabling the simultaneous detection of neurons expressing both transgenes, GFP and TG6.

B) Western blotting of transduced TG6-WT and TG6 mutants in primary cortical neurons. TG6-WT and TG6 mutants were detected with anti-V5 antibody. Graph, mean  $\pm$  SEM, n = 3, \*\*p<0.01, 1-way ANOVA with Tukey's *post hoc* test.

### Supplementary Figure 5 N-terminal mCherry tag does not affect expression and activity of TG6.

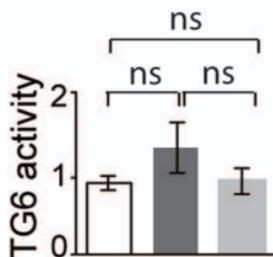
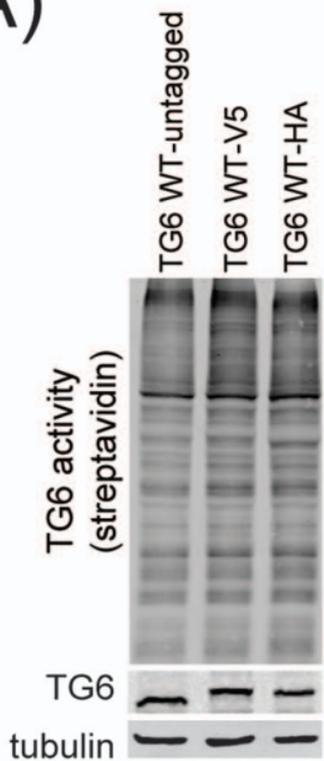
A) Fusion of mCherry to the N-terminus of TG6 does not affect TG6 expression and activity, whereas fusion of mCherry to the C-terminus significantly reduced its enzymatic activity. Graph, mean  $\pm$  SEM, n = 3, \*p<0.05; 1-way ANOVA with Tukey's *post hoc* test.

B) mCherry-TG6-R111C showed decreased activity compared to mCherry-TG6-WT. Graph, mean  $\pm$  SEM, n = 3, \*p<0.05; Student's t-test.

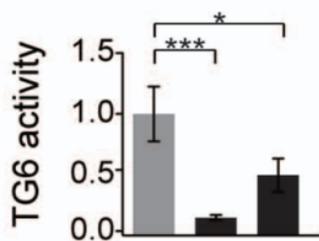
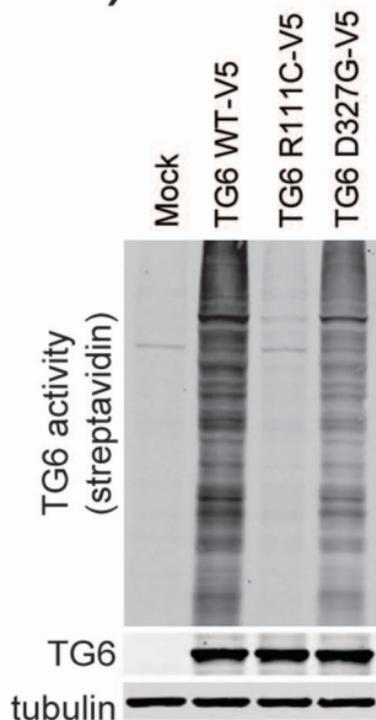


Supplementary Figure 1

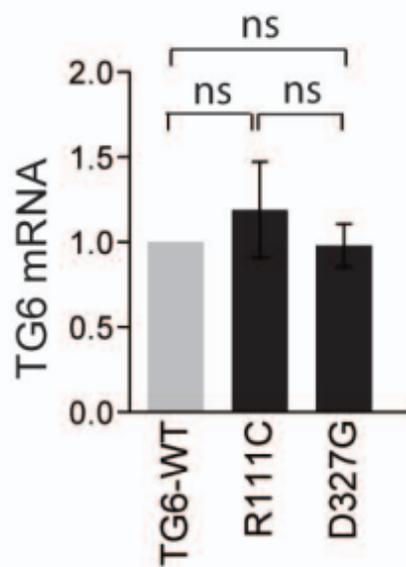
A)



B)

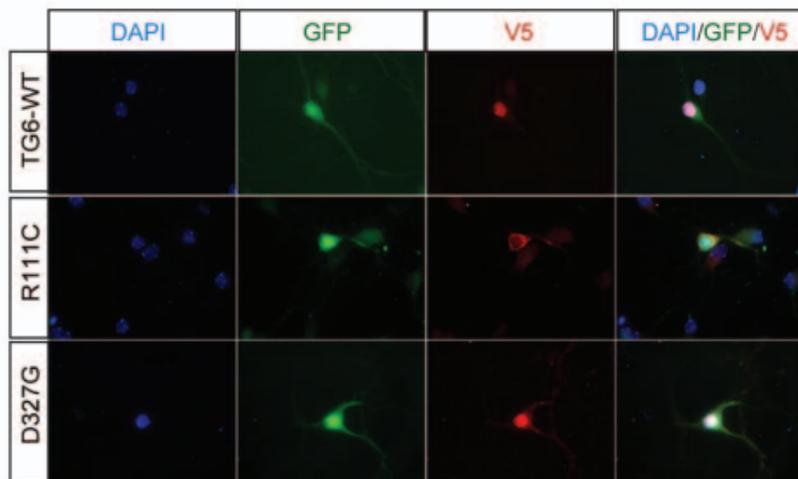


Supplementary Figure 2

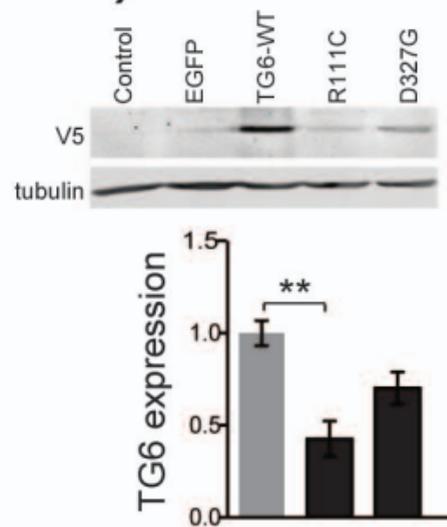


Supplementary Figure 3

A)

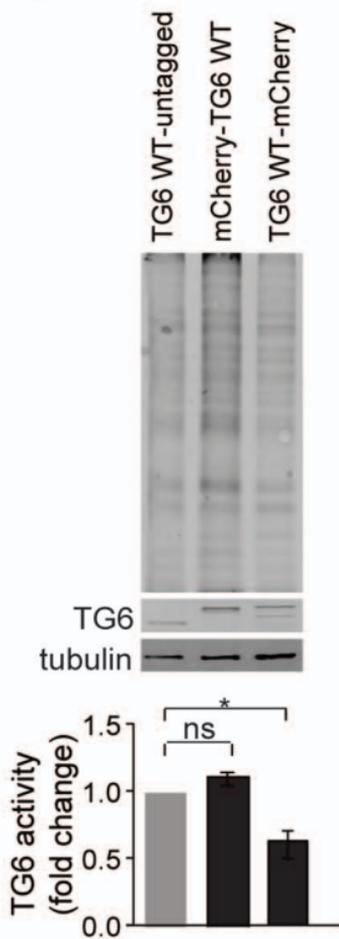


B)



Supplementary Figure 4

A)



B)

