Supplemental Figures and Methods

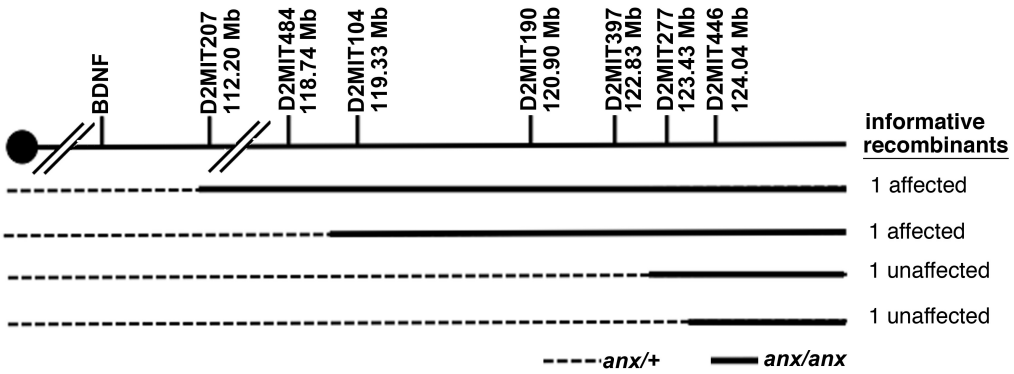
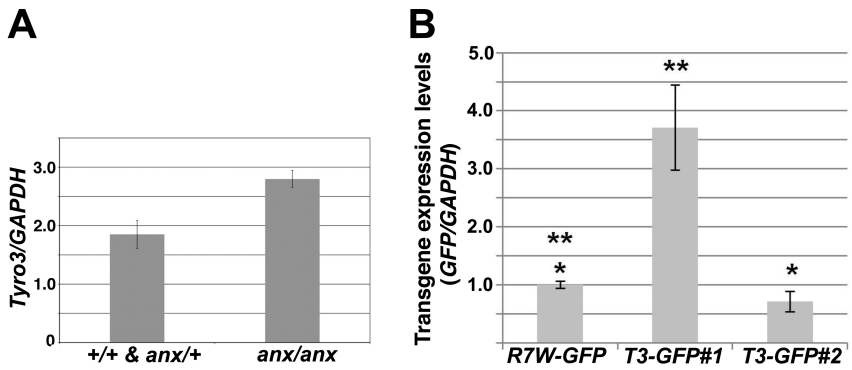


Figure S1. Refinement of the *anx* critical interval by recombinant mapping
Meiotic mapping was performed on 335 progeny from *anx*/+ intercrosses with the SSLPs shown. 5 informative recombinants and their phenotypes are shown. Two recombinants refined the critical interval to a 3.2Mb region between D2Mit484 and D2Mit190.



(Unpaired t-test:
** p = 0.046, *p = 0.302)

Figure S2. *Tyro3* RNA levels in *anx/anx* brains at P21 and levels of Tyro3-GFP and R7W-Tyro3-GFP transgene expression in +/+ brains at P21.
(A) Levels of Tyro3 RNA normalized relative to GAPDH were determined for *anx*/*anx*, *anx*/+ and +/+ brains at P21 by qRT-PCR. n=3 for each genotype. Error bars indicate the standard error of the mean. No significant differences in *Tyro3* levels were seen between these three genotypes.
(B) *Tyro3-GFP* expression levels relative to *R7W-Tyro3-GFP* in adult +/+ brains. *Tyro3-GFP* expression levels in the *Tyro3-GFP#1* line (T3-1) were significantly higher than the *R7W-Tyro3-GFP* line (R7W), as analyze by qRT-PCR. No significant difference was observed in expression levels between the *Tyro3-GFP#2* line (T3-2) and the *R7W-Tyro3-GFP* line (R7W). GFP expression from each sample (n=3) was repeated in triplicate and normalized to *GAPDH*. Regardless of difference in expression level both T3-1 and T3-2 Tyro3-GFP lines showed partial rescue of *anx* phenotypes while R7W-Tyro3-GFP did not.

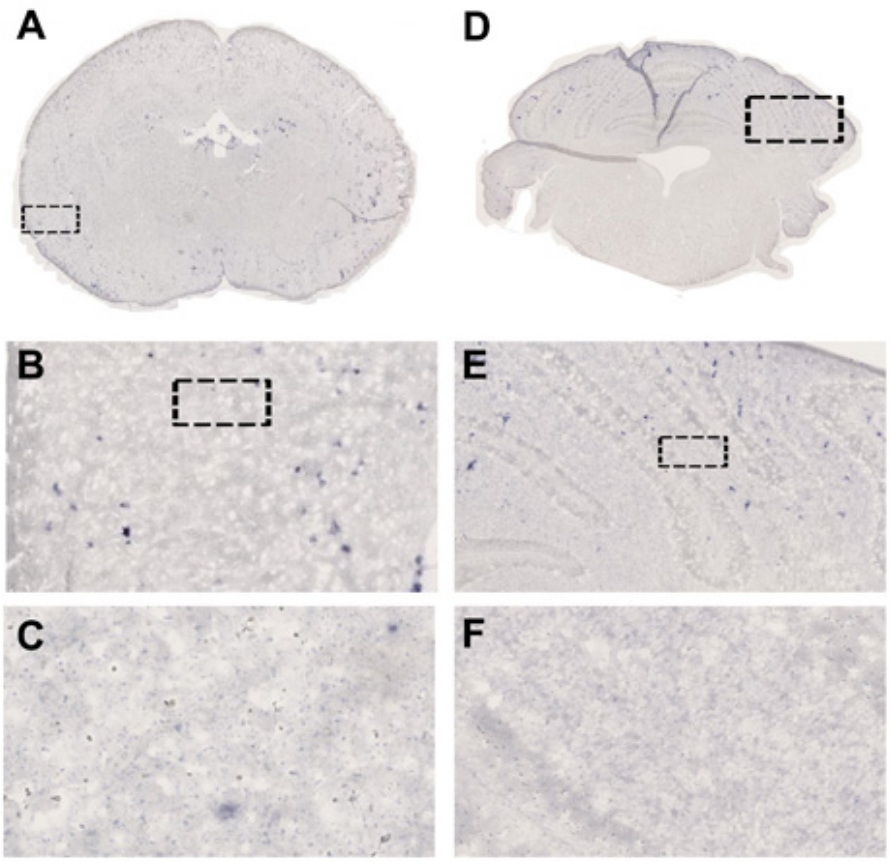


Figure S3. RNA in situ hybridization with *Tyro3* sense probe does not detect appreciable signal. View of complete representative sections are shown in **A** and **D**. Magnified regions of cortex from **A** are shown in **B** and **C**. Magnified regions of cerebellum from **D** are shown in **E** and **F**. Dashed boxes in **A** and **D** indicate region magnified in **B** and **E**, respectively. Dashed boxes in **B** and **E** indicate region magnified in **C** and **F**, respectively.

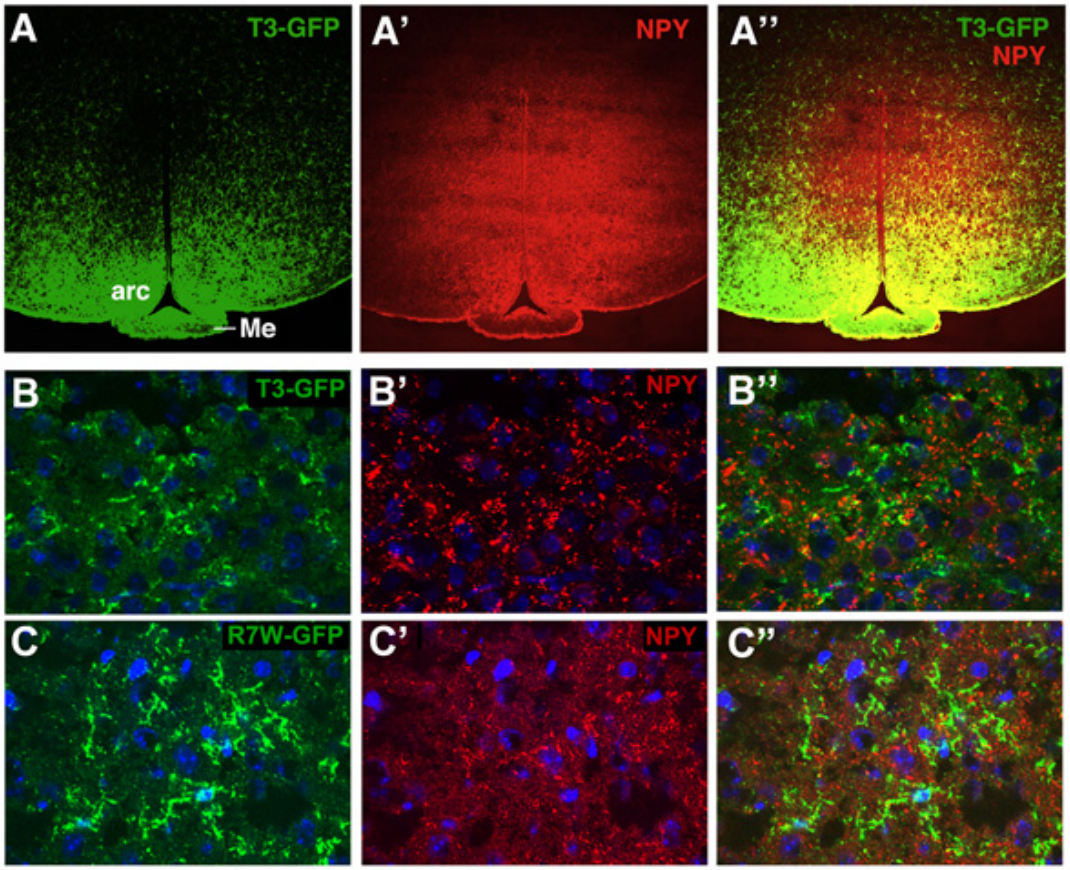


Figure S4. ***Tyro3-GFP*** **expression in the hypothalamus.**
Immunofluorescence with an (**A**) anti-GFP (green) and (**A'**) anti-Npy (red) antibodies detects Tyro3-GFP in the arcuate nucleus (arc) and median eminence (Me) of Tyro3-GFP (line #1); +/+ brains at P21 (10x magnification). The merged image is shown in (**A''**). Tyro3-GFP (green) and Npy expression, as detected by immunofluorescence with an anti-Npy antibody (red), overlap in cells lining the inside and outside of the median eminence and in the arcuate nucleus. High magnification views of the arcuate nucleus from (**B-B"**) Tyro3-GFP; +/+ and (**C-C'**) R7W-Tyro3-GFP; +/+ mice show co-expression of tagged transgenes with Npy-expressing cells. However, Tyro3-GFP and R7W-Tyro3-GFP containing subcellular domains within cells appear separate from those staining for Npy, as would be expected for a membrane associated tyrosine kinase receptor and a secreted neuropeptide, such as Npy. T3-GFP refers to Tyro3-GFP; R7W-GFP refers to R7W-Tyro3-GFP.

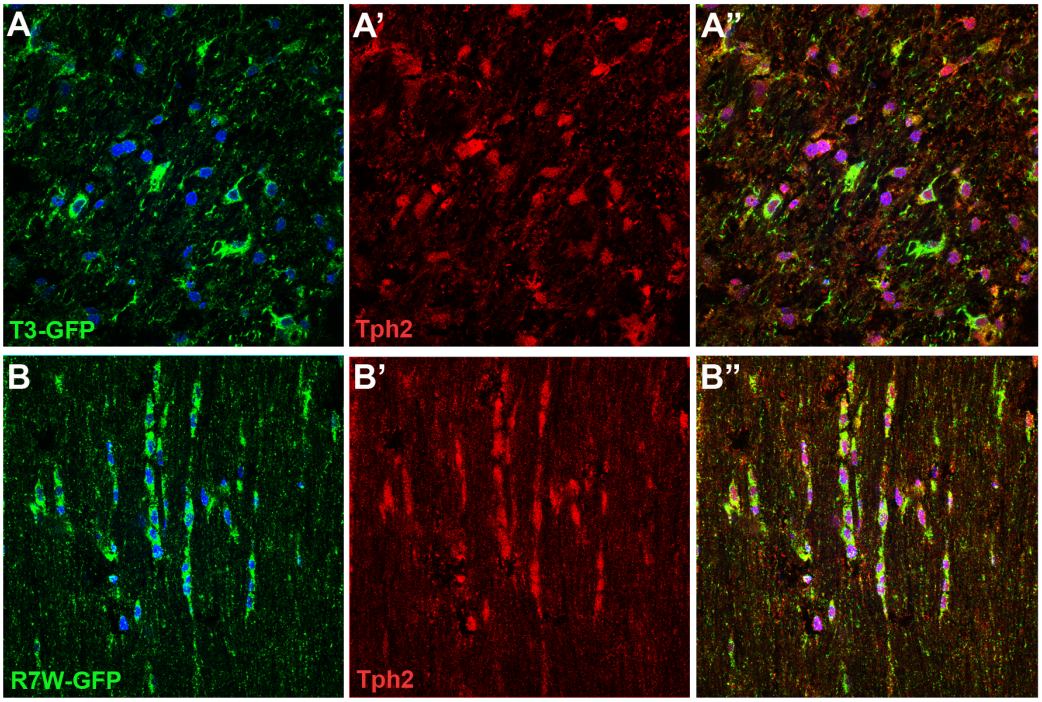


Figure S5. Tyro3-GFP and R7WTyro3-GFP are expressed in 5-HT neurons.
Representative sections through P10 hindbrains of (**A-A"**) Tyro3-GFP;+/+ and (**B-B"**) R7WTyro3-GFP;+/+ are shown. Immunofluorescence with (**A**, **B**) anti-GFP (green) and (**A'**, **B'**) anti-Tph2 (red) antibodies detects expression of *Tyro3-GFP* and *R7W-Tyro3-GFP* transgenes in 5-HT neurons. (**A"**, **B"**) show merged images.

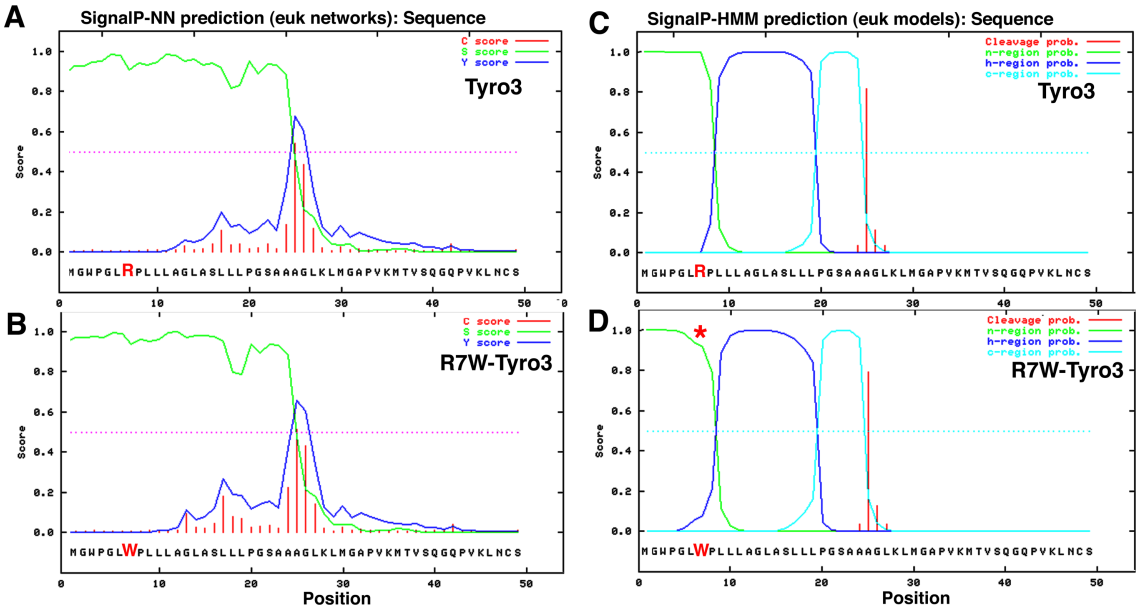


Figure S6. Comparison of Signal Sequence Predictions from SignalP Analysis. The first 49 amino acids of the normal Tyro3 and R7W-Tyro3 were examined using SignalP (Nielsen and Krogh, 1998) to determine any aberrations resulting from the arginine-to-tryptophan conversion. The signal sequence cleavage site at amino acid 25 was predicted to be the same in (**A**) normal Tyro3 and (**B**) R7W-Tyro3. Comparison of the plots of the **(C)** normal and **(D)** mutant protein reveals an extremely slight deviation between the n- and h-region in the mutant protein marked with an asterisk.

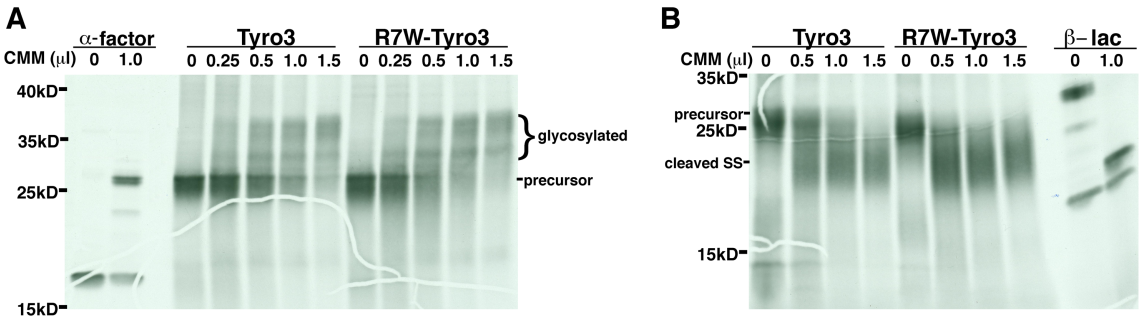


Figure S7. Tyro3 and R7W-Tyro3 are processed equivalently *in vitro*. *In vitro* transcription/translation (TnT) of Tyro3 and R7W-Tyro3 templates containing all eight predicted N-glycosylation sites coupled with the addition of canine pancreatic microsomal membranes (CMM) and subsequent addition of endoglycosidase H was used to examine the co-translational processing of Tyro3 and R7W-Tyro3. (**A**) *In vitro* TnT coupled with the addition of CMM resulted in the synthesis and processing (glycosylation) of both Tyro3 and R7W-Tyro3 as indicated by the increase in protein size. (**B**) Removal of glycosylation with endoglycosidase H revealed that both Tyro3 and R7W-Tyro3 signal sequences had been cleaved as detected by the equivalent decrease in protein size relative to the precursor protein. Glycosylation of alpha-factor from S. cerevisiae and Beta-lactamase signal sequence cleavage serve as positive controls.

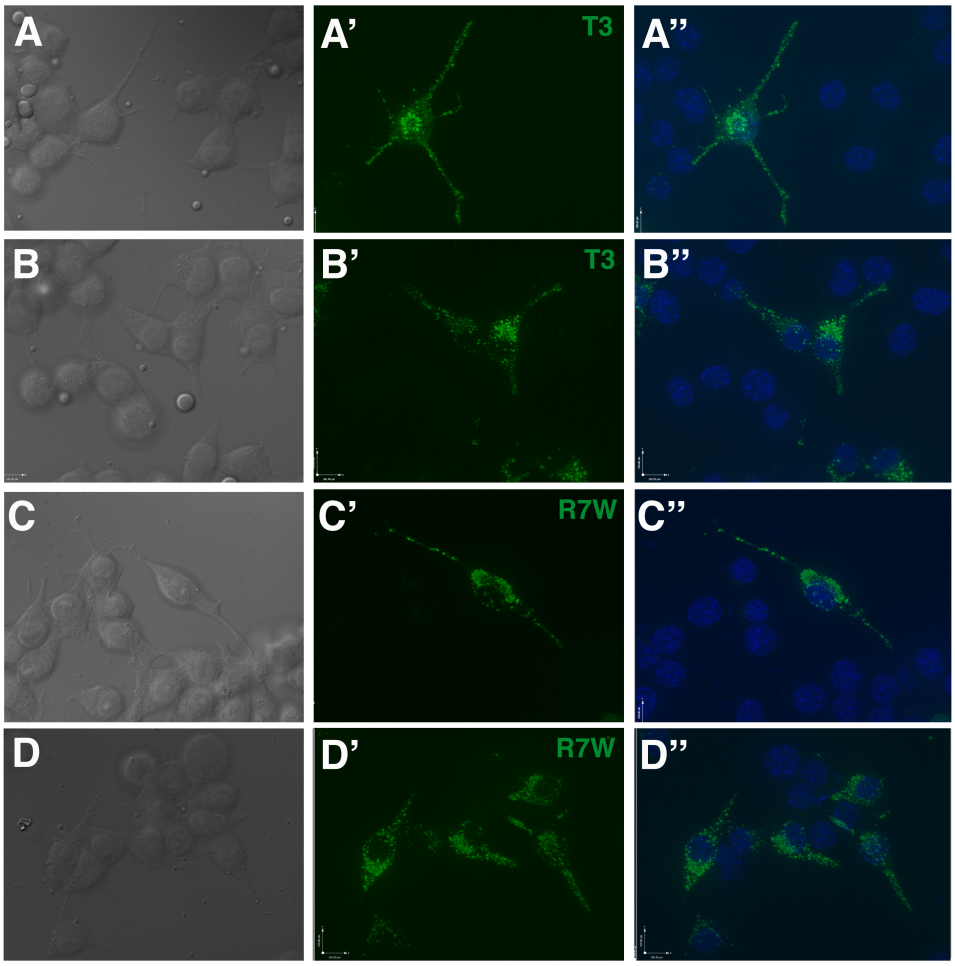


Figure S8. Tyro3-GFP and R7W-Tyro3-GFP are localized equivalently in transiently transfected Neuro2A cells.
(**A-B''**)Tyro3-GFP and (**C-D''**)R7W-Tyro3-GFP proteins are distributed throughout the soma and along processes of transiently transfected Neuro2A cells indistinguishably from each other. **A - D** show DIC views; **A' - D'** show GFP fluorescence of the tagged proteins; and **A" - D"** show GFP fluorescence of tagged proteins in conjunction with DAPI stained nuclei.

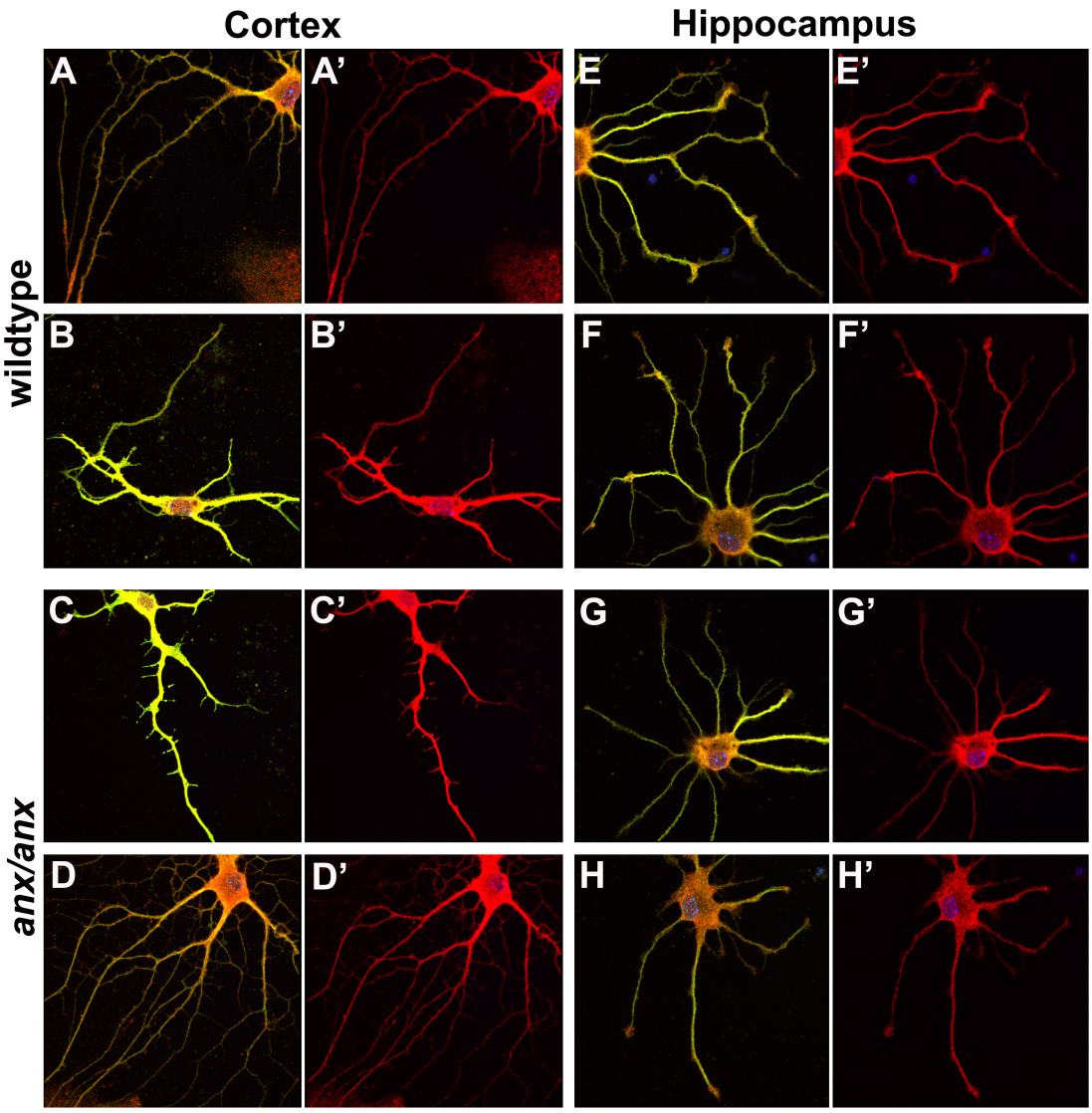


Figure S9. Tyro3 and R7W-Tyro3 distribution in cultured cortical and hippocampal neurons.
Protein localization of Tyro3 and R7W-Tyro3 was examined in primary cultures of (**A**, **B**, **E**, **F**) +/+ and (**C**, **D**, **G**, **H**) *anx/anx* cultured neurons after 10 days in culture (DIV10) by immunofluorescence with an anti-Tyro3 (red). Dendrites were labeled by immunofluorescence with anti-MAP2 antibody (green). **A-D**: cortical neurons; **E-H**: hippocampal neurons. Anti-Tyro3 immunofluorescence can be seen throughout neuronal processes in all cases.