Entorhinal Cortical Neurons Are the Primary Targets of FUS Mislocalization and Ubiquitin Aggregation in FUS Transgenic Rats

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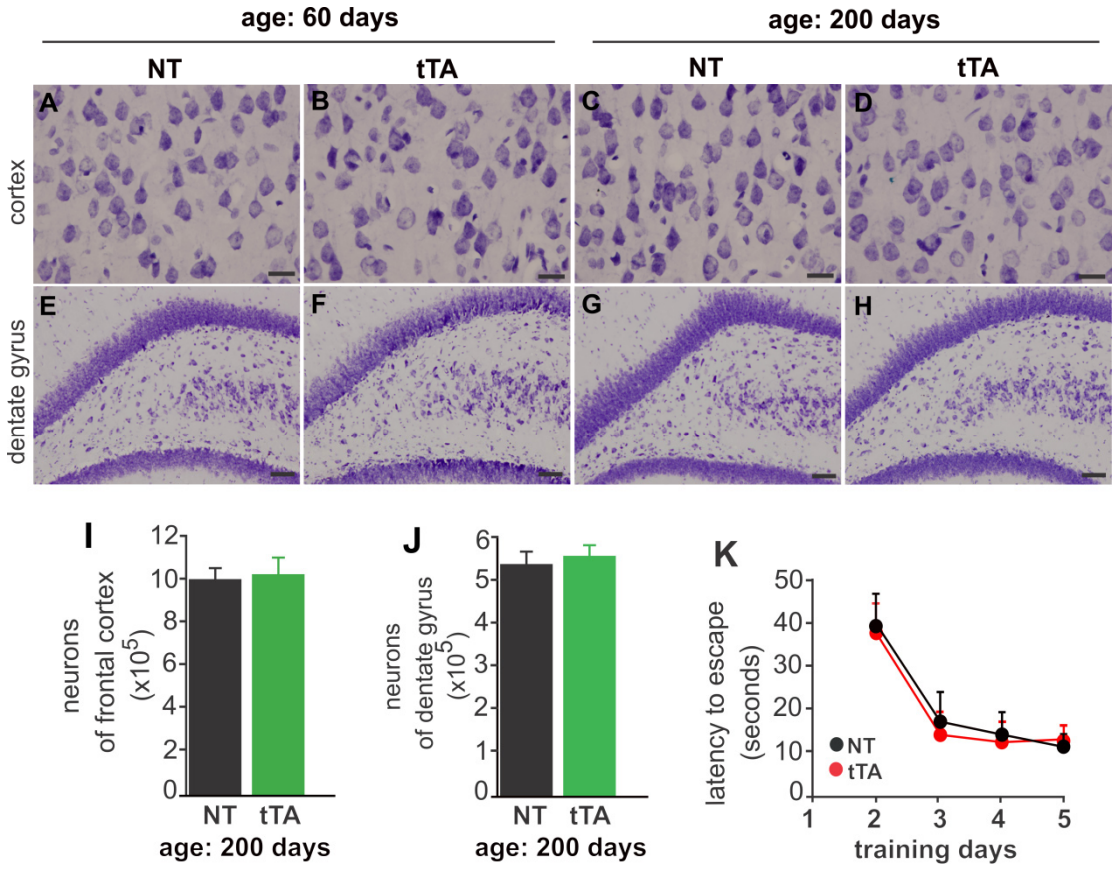

Figure S1: Camk2a-tTA transgenic rats possess the normal ability in learning and memory. (**A-H**) Cresyl violet staining shows the structure of frontal cortex (A-D) and dentate gyrus (E-H) taken from Camk2-tTA transgenic rats (tTA) or the nontransgenic littermates (NT). Scale bars: A-D, 20 μm; E-H, 80 μm. (**I, J**) Stereological cell counting reveals no difference in the number of cortical (I) and hippocampal (J) neurons between paired NT and tTA littermates. Data are means + SEM (n =4). (**K**) Barnes maze assay reveals no difference in spatial learning and memory between paired NT and tTA littermates at the age of 200 days. Data are means + SEM (n = 6).

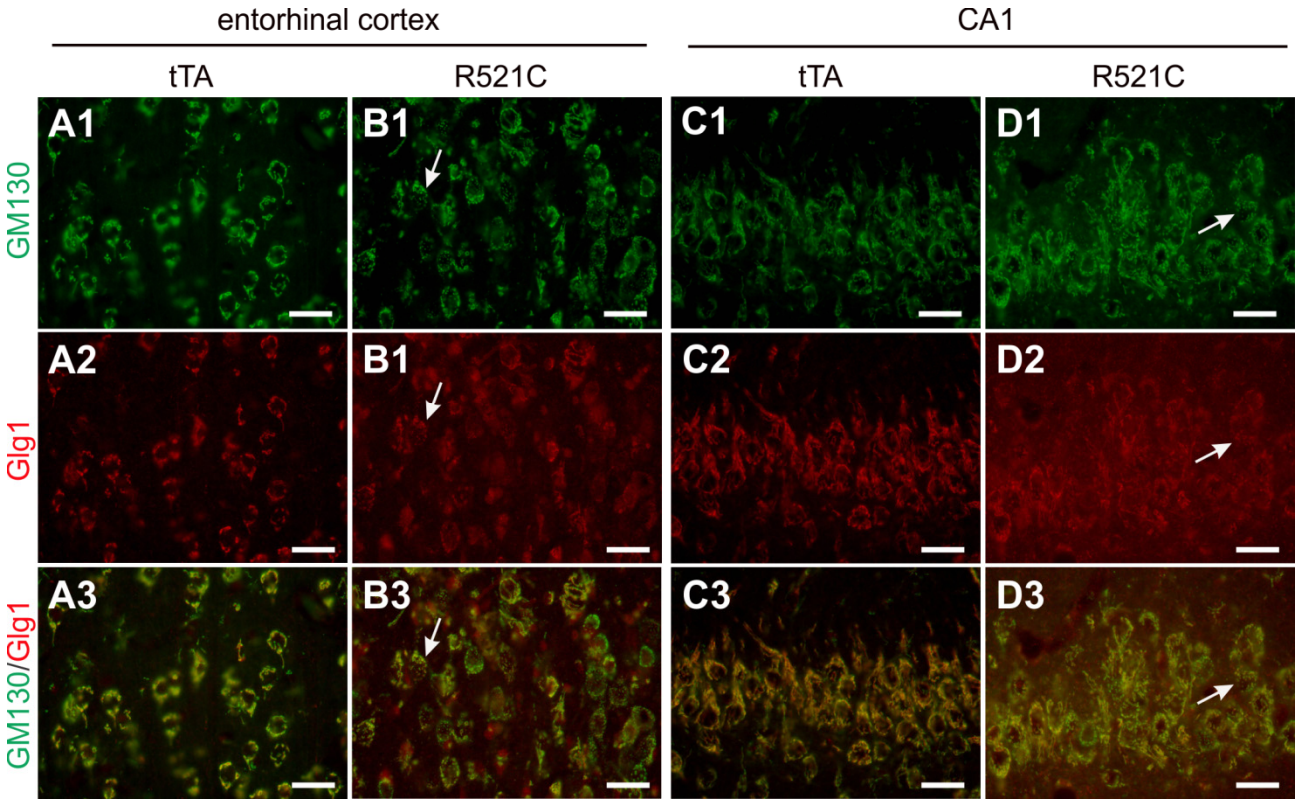

Figure S2: Golgi complex is fragmented in mutant FUS transgenic rats. (**A-D**) Double-labeling fluorescence staining reveals that both Cis (stained of GM130) and Trans (stained of Glg1) Golgi complexes were fragmented in Camk2a-tTA/TRE-FUS^{R521C} double transgenic rat (R521C) as compared to Camk2a-tTA single transgenic rat (tTA). Golgi structure was examined under fluorescence microscope for the rats of 50 days old. Arrows point to representative cells with fragmented Golgi. Note that multiple cells (B1-3 and D1-3) display fragmented Golgi in R521C rats. Scale bars: J-O, 30 μm.

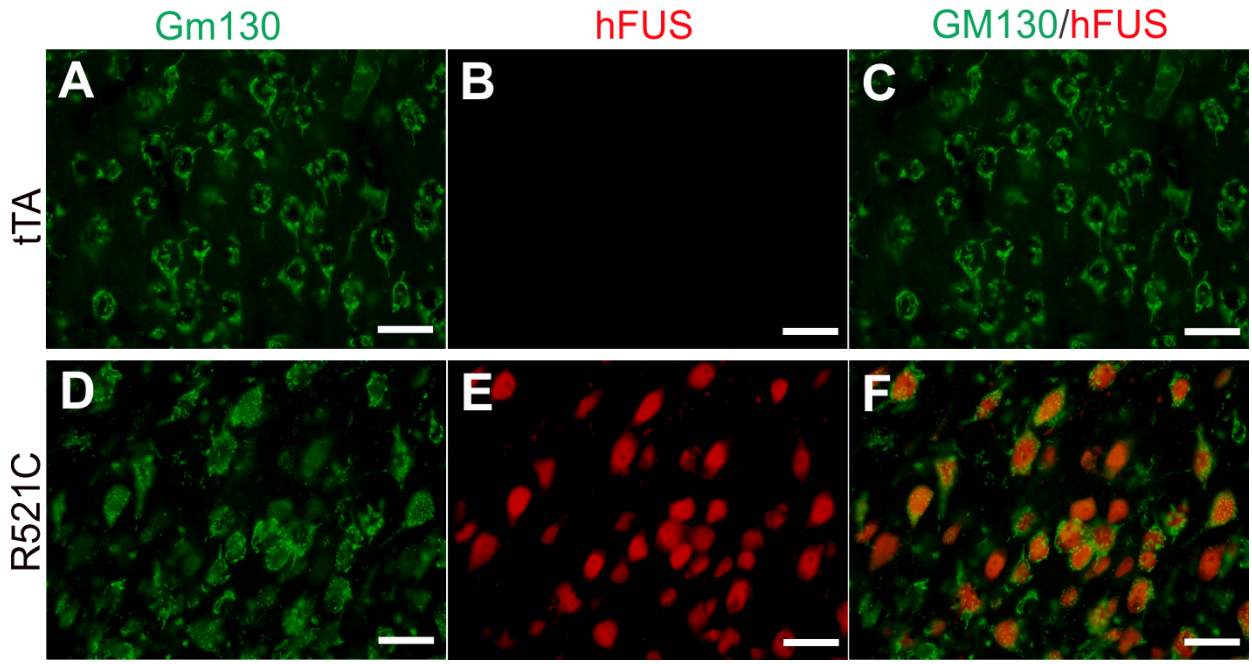

Figure S3: Overexpression of mutant human FUS leads to Golgi fragmentation. (**A-F**) Fluorescence staining reveals that only the cells expressing mutant human FUS (R521C) displayed fragmented Golgi (stained of GM130). Images of the entorhinal cortex (A-F) were taken of Camk2a-tTA/TRE-FUS^{R521C} double transgenic rat (R521C) or Camk2a-tTA single transgenic rat (tTA) at the age of 50 days. All scale bars: 30 μm.

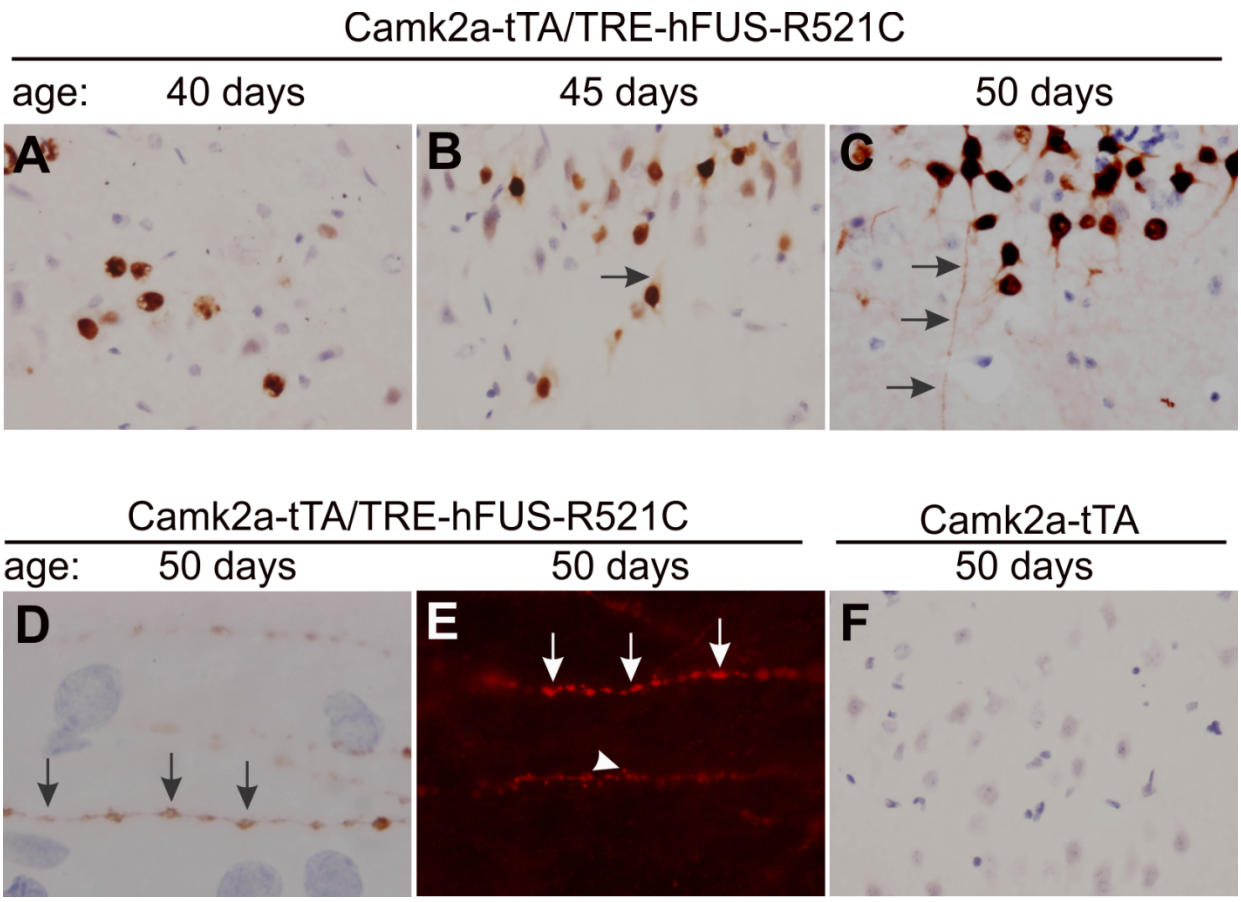

Figure S4: Mutant human FUS gradually accumulates in the neurites and dendritic spines in rats. (**A-C**) Immunostaining of human FUS reveals that mutant human FUS gradually accumulated in the neurites (arrows) in the entorhinal cortex as disease was progressing. Camk2a-tTA/TRE-FUS^{R521C} double transgenic rats were deprived of Dox at the age of 30 days so that human FUS transgene was activated subsequently. (**D, E**) Images of high magnification show that human FUS was accumulated in the neurites (arrows) and in the dendritic spines (arrowhead). (**F**) The antibody recognizing human FUS did not cross-react with rat's FUS protein in the tTA single transgenic rat.

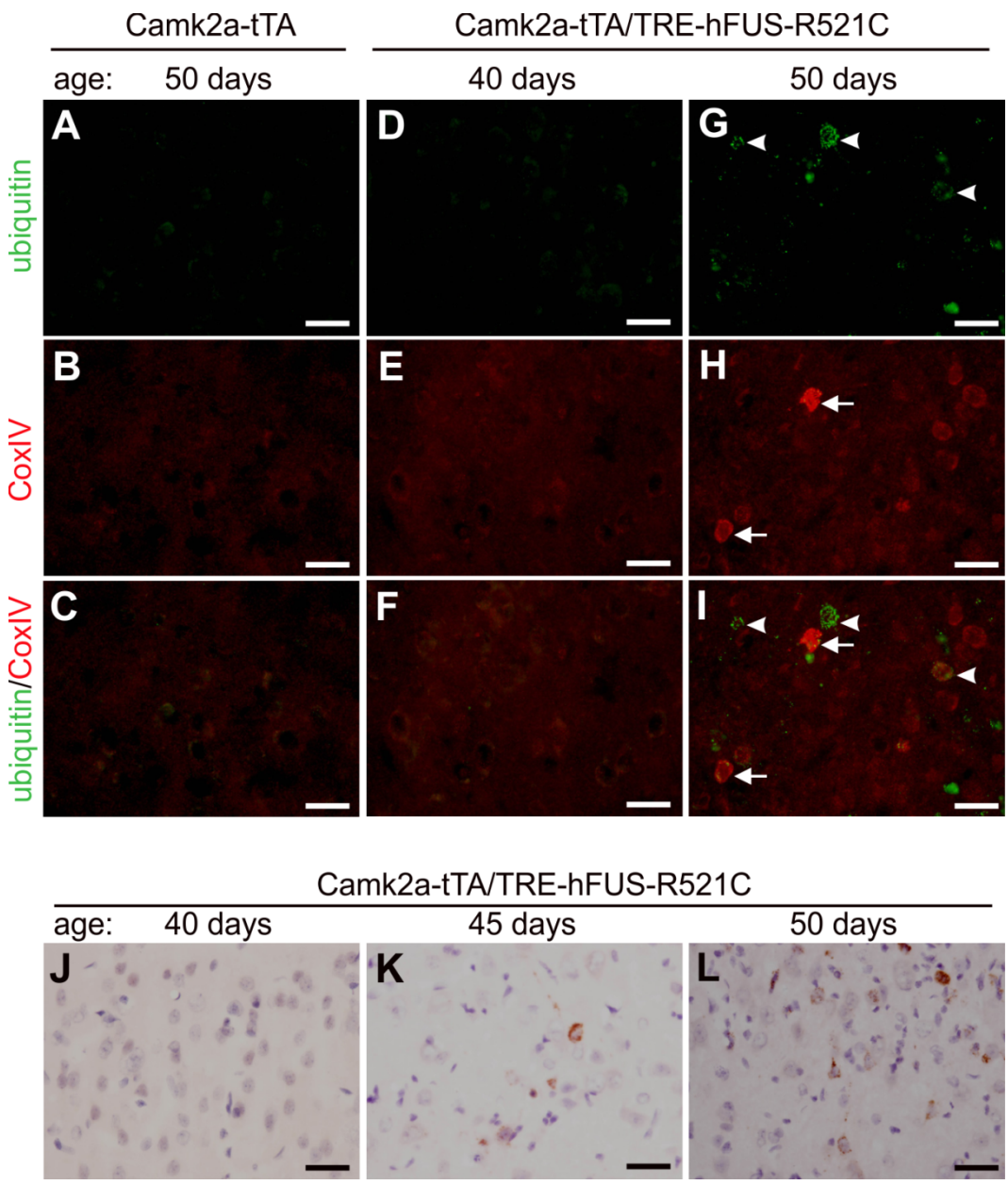

Figure S5: Ubiquitin gradually accumulates in the entorhinal cortical neurons of mutant FUS transgenic rats. (**A-I**) Fluorescence staining reveals that ubiquitin and CoxIV were accumulated in the neurons of entorhinal cortex as disease was progressing. Note that CoxIV staining was marginally detectable and evenly distributed in the cortical neurons of the control rat (Camk2a-tTA). (**J-L**) Immunostaining showed that ubiquitin was quickly accumulated in the neurons of entorhinal cortex in FUS transgenic rats. Coronal sections (14 μm) of the forebrain were immunostained of human FUS and were counterstained with hematoxylin to display the nuclei. All scale bars: 30 μm.