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SUPPLEMENTARY INFORMATION

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C9ORF72-ALS/FTD-associated poly(GR) binds Atp5a1 and compromises mitochondrial function in vivo

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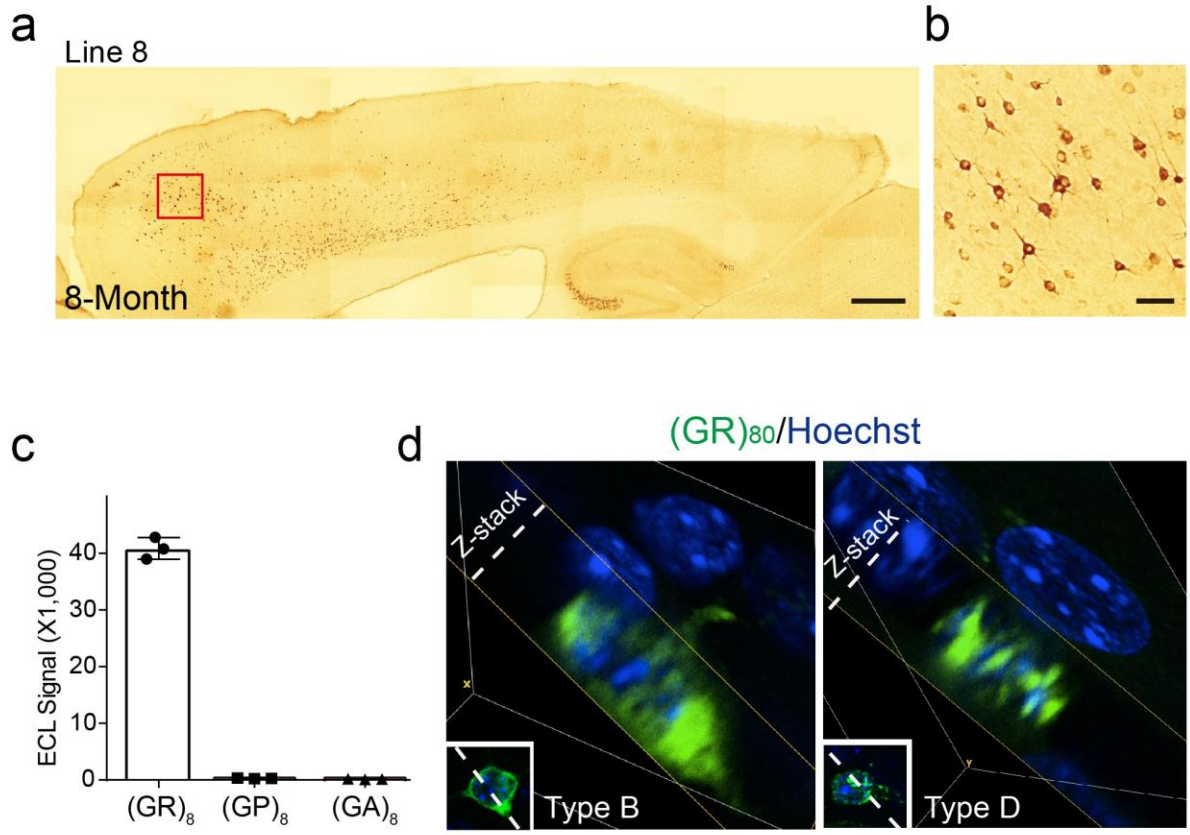
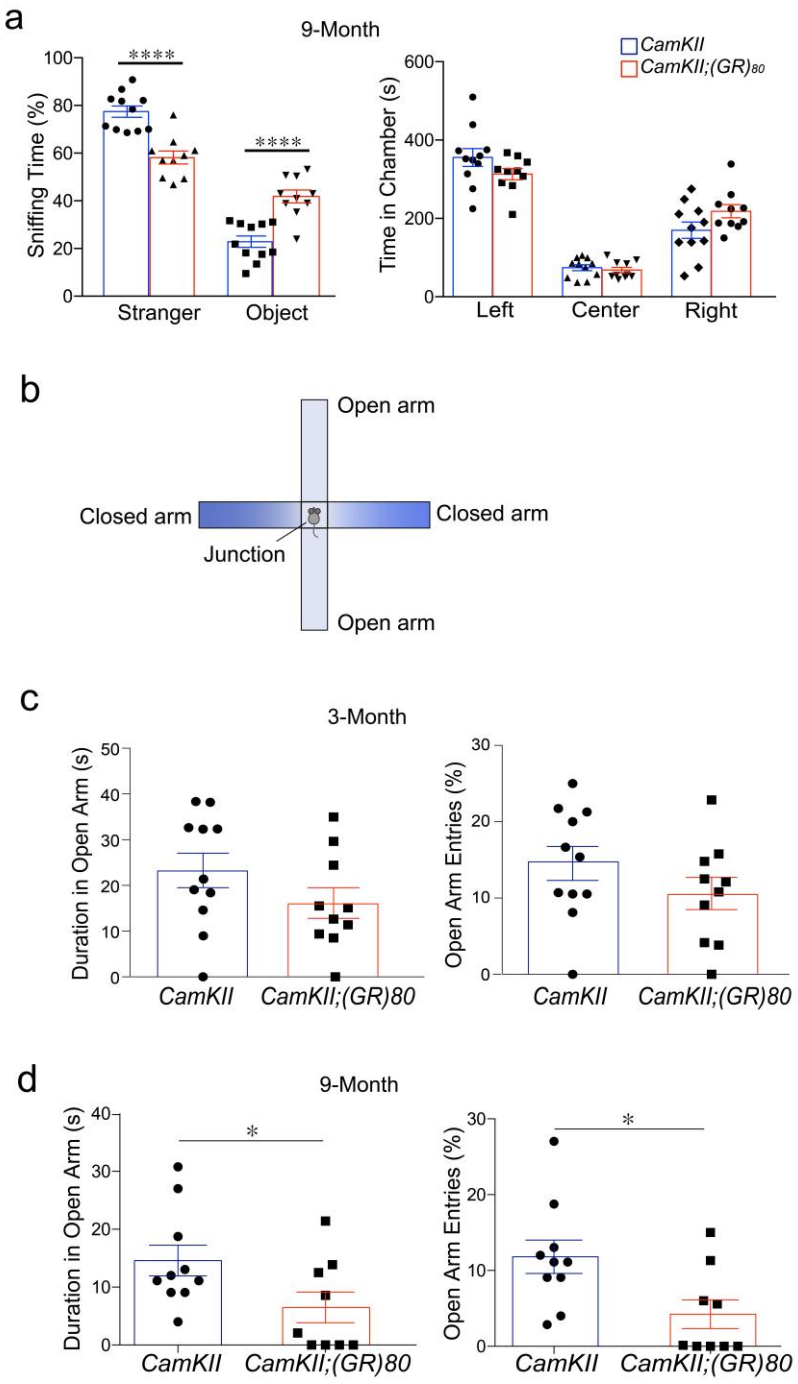


Supplementary Figure 1

Poly(GR) expression in *CamKII;(GR)80* mice

a, A representative image showing preferential accumulation of poly(GR) in frontal cortex relative to other cortical regions of line 8 *CamKII;(GR)80* mice at 8 months of age, selected from three independent immunostaining experiments. Scale bar: 500 µm. **b,** Enlarged image of the area highlighted by the red square in Panel a. Scale bar: 50 µm. **c,** The newly made rabbit polyclonal poly(GR) antibody is specific to (GR)8 and does not react with (GP)8 or (GA)8. The values are mean ± s.d. by one-way ANOVA with Tukey's post hoc analysis for multiple comparisons: $F(2,7) = 1372$, $P < 0.0001$ for (GR)8 vs. (GP)8, $P < 0.0001$ for (GR)8 vs. (GA)8, $P = 0.9924$ for (GP)8 vs. (GA)8. (GR)8 = 4118.67 ± 1912.87, n = 3; (GP)8 = 254 ± 41.8, n = 3; (GA)8 = 147.66 ± 36.11 from three independent ELISA experiments. **d,** A representative z-stack image of poly(GR) nuclear localization in Type B and Type D neurons of *CamKII;(GR)80* mice at 8 months of age selected from 5 images. This experiment was repeated three time.

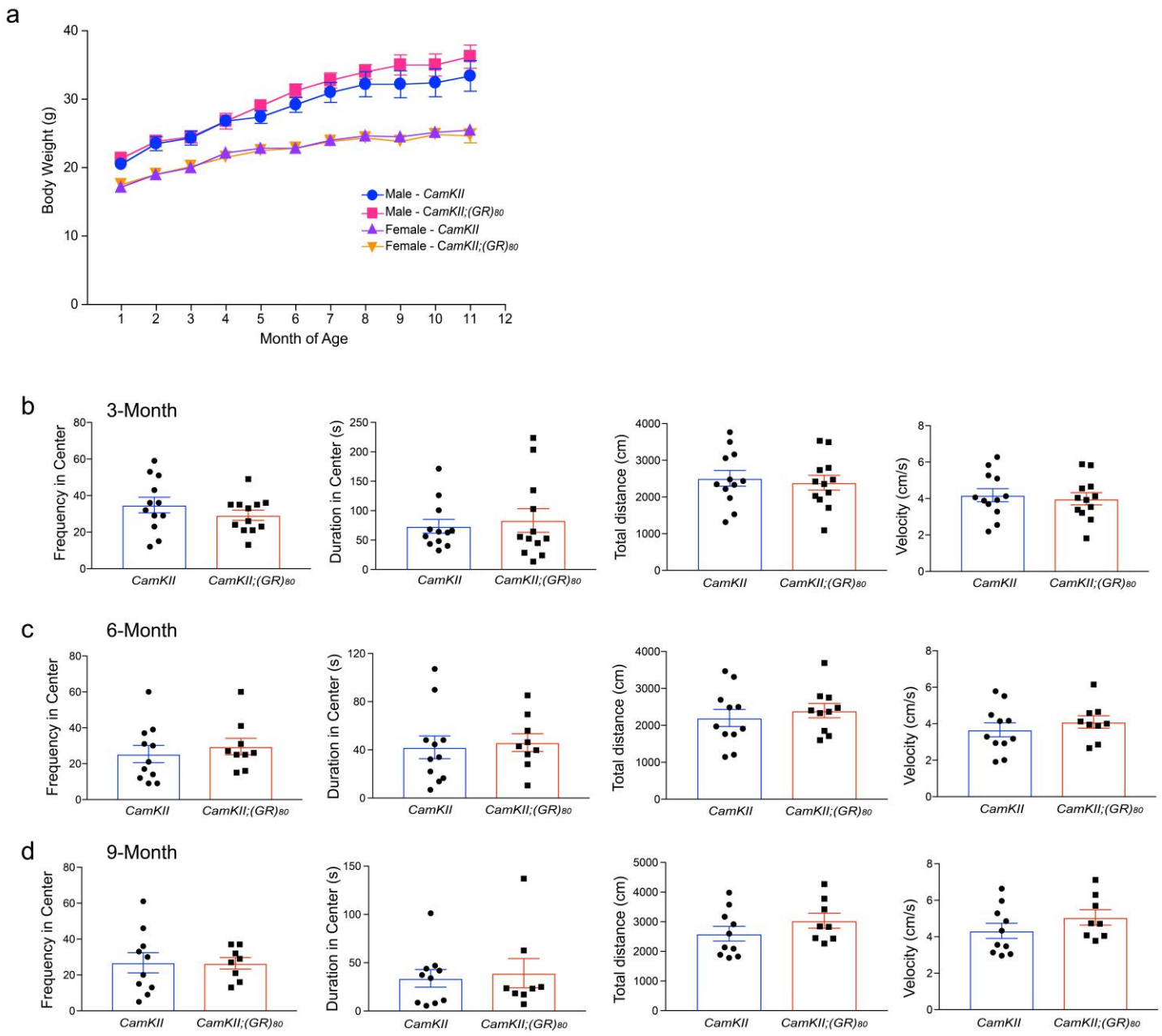


Supplementary Figure 2

Age-dependent behavioral phenotypes of *CamKII* and *CamKII;(GR)80* mice

a, The three-chamber social interaction test for *CamKII* and *CamKII;(GR)80* mice at 9 months of age. *CamKII*:Stranger = 77.43 ± 2.40, n = 11 mice; *CamKII*;(GR)80:Stranger = 58.16 ± 2.72, n = 10 mice; *CamKII*:Object = 22.57 ± 2.40, n = 11 mice; *CamKII*;(GR)80:Object = 41.84 ± 2.72, n = 10 mice; *CamKII*:Left=355.7 ± 22.78, n = 11 mice; *CamKII*;(GR)80:Left = 313 ± 14.28, n = 10 mice; *CamKII*:Center = 74.28 ± 7.59, n = 11 mice; *CamKII*;(GR)80:Center = 68.30 ± 6.99, n = 10 mice; *CamKII*:Right = 169.9 ± 20.69, n = 11 mice; *CamKII*;(GR)80:Right = 218.4 ± 16.81, n = 10 mice. Values are means ± s.e.m. by Student's t test, two-sided: F(1,19) = 1.17, *P* <

0.0001 for *CamKII*:Stranger vs. *CamKII*;(GR)80:Stranger, F(1,19) = 1.17, *P* < 0.0001 for *CamKII*:Object vs. *CamKII*;(GR)80:Object, F(1, 19) = 2.8, *P* = 0.1383 for *CamKII*:Left vs. *CamKII*;(GR)80:Left, F(1, 19) = 1.3, *P* = 0.5791 for *CamKII*:Center vs. *CamKII*;(GR)80:Center, F(1, 19) = 1.67, *P* = 0.0882 for *CamKII*:Right vs. *CamKII*;(GR)80:Right. **b,** Schematic of elevated plus maze test. *CamKII* or *CamKII;(GR)80* mice were placed in the center of the elevated plus maze and allowed to explore for 5 min. **c,** Time spent (left graph) and percentage of entries (right graph) in the open arm of the elevated plus maze by *CamKII* and *CamKII;(GR)80* mice at 3 months of age. *CamKII* = 23.20 ± 3.78, n = 11 mice; *CamKII;(GR)80* = 15.92 ± 3.36 in the left graph, n = 10 mice; *CamKII* = 14.54 ± 2.22, n = 11 mice; *CamKII;(GR)80* = 10.46 ± 2.12 in the right graph, n = 10 mice. Values are mean ± s.e.m. by two-sided Student's t test: F(1, 19) = 5.98, *P* = 0.1636 for *CamKII* vs. *CamKII;(GR)80* in the left graph, F(1, 19) = 1.21, *P* = 0.2008 for *CamKII* vs. *CamKII;(GR)80* in the right graph. **d,** Time spent (left graph) and percentage of entries (right graph) in the open arm of the elevated plus maze by *CamKII* and *CamKII;(GR)80* mice at 9 months of age. Each dot represents one mouse. *CamKII* = 14.60 ± 2.67, n = 10 mice; *CamKII;(GR)80* = 6.50 ± 2.65 in the left graph, n = 9 mice; *CamKII* = 10.00 ± 2.21, n = 10 mice; *CamKII;(GR)80* = 4.23 ± 1.90 in the right graph, n = 9 mice. Values are mean ± s.e.m. by two-sided Student's t test: F(1, 17) = 1.2, *P* = 0.0464 for *CamKII* vs. *CamKII;(GR)80* in the left graph, F(1, 17) = 1.5, *P* = 0.0196 for *CamKII* vs. *CamKII;(GR)80* in the right graph.

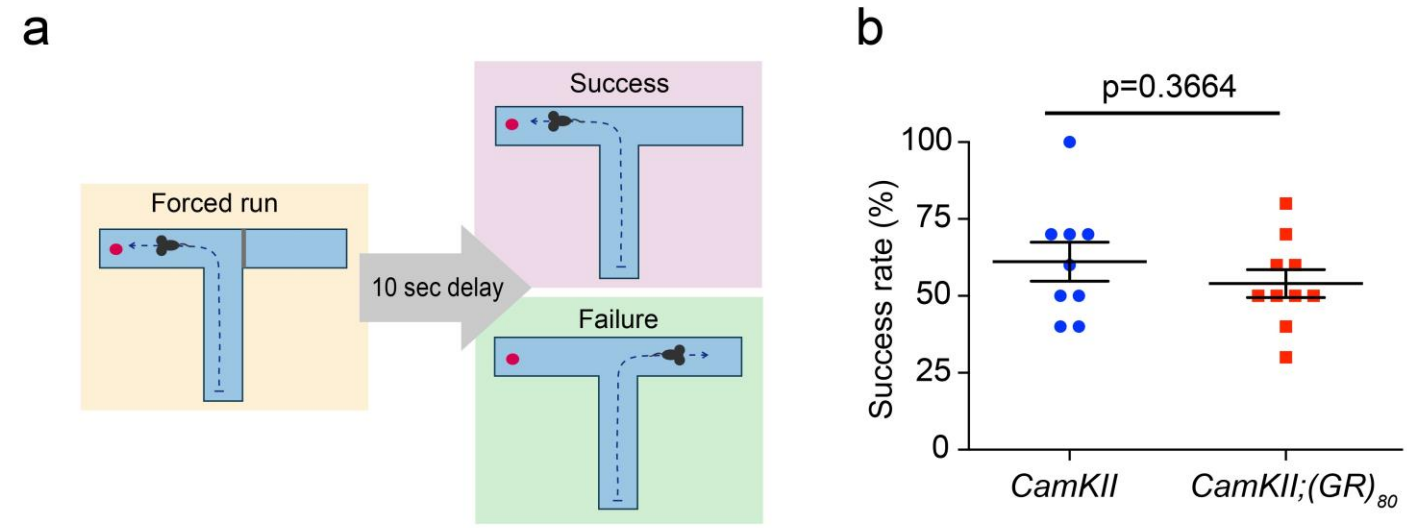


Supplementary Figure 3

Body weight and open field test of locomotor activity of *CamKII* and *CamKII;(GR)80* mice

a, The body weight of five mice of each genotype and sex was measured monthly. There was no difference between *CamKII* and *CamKII;(GR)80* mice. *CamKII*:male:1 month = 20.50 ± 0.85, n = 6 mice; *CamKII*;(GR)80:male:1 month = 21.33 ± 0.80, n = 6 mice; *CamKII*:male:2 months = 23.50 ± 1.02, n = 6 mice; *CamKII*;(GR)80:male:2 months = 23.83 ± 0.79, n = 6 mice; *CamKII*:male:3 months = 24.33 ± 1.02, n = 6 mice; *CamKII*;(GR)80:male:3 months = 24.50 ± 0.92, n = 6 mice; *CamKII*:male:4 months = 26.80 ± 0.73, n = 5 mice; *CamKII*;(GR)80:male:4 months = 26.80 ± 1.16, n = 5 mice; *CamKII*:male:5 months = 27.4 ± 0.93, n = 5 mice; *CamKII*;(GR)80:male:5 months = 29.00 ± 0.71, n = 4 mice; *CamKII*:male:6 months = 29.20 ± 1.11, n = 5 mice; *CamKII*;(GR)80:male:6 months = 31.25 ± 1.03, n = 4 mice; *CamKII*:male:7 months = 31.00 ± 1.45, n = 5 mice; *CamKII*;(GR)80:male:7 months = 32.75 ± 1.11, n = 4 mice; *CamKII*:male:8 months = 32.20 ± 1.83, n = 5 mice; *CamKII*;(GR)80:male:8 months = 34.00 ± 1.08, n = 4 mice; *CamKII*:male:9 months = 32.20 ± 1.98, n = 5 mice; *CamKII*;(GR)80:male:9 months = 35.00 ± 1.47, n = 4 mice; *CamKII*:male:10 months = 32.40 ± 2.06, n = 5 mice; *CamKII*;(GR)80:male:10 months = 35.00 ± 1.63, n = 4 mice; *CamKII*:male:11 months = 33.40 ± 2.23, n = 5 mice; *CamKII*;(GR)80:male:11 months = 36.25 ± 1.70, n = 4 mice; *CamKII*:female:1 month = 17.17 ± 0.31, n = 6 mice;

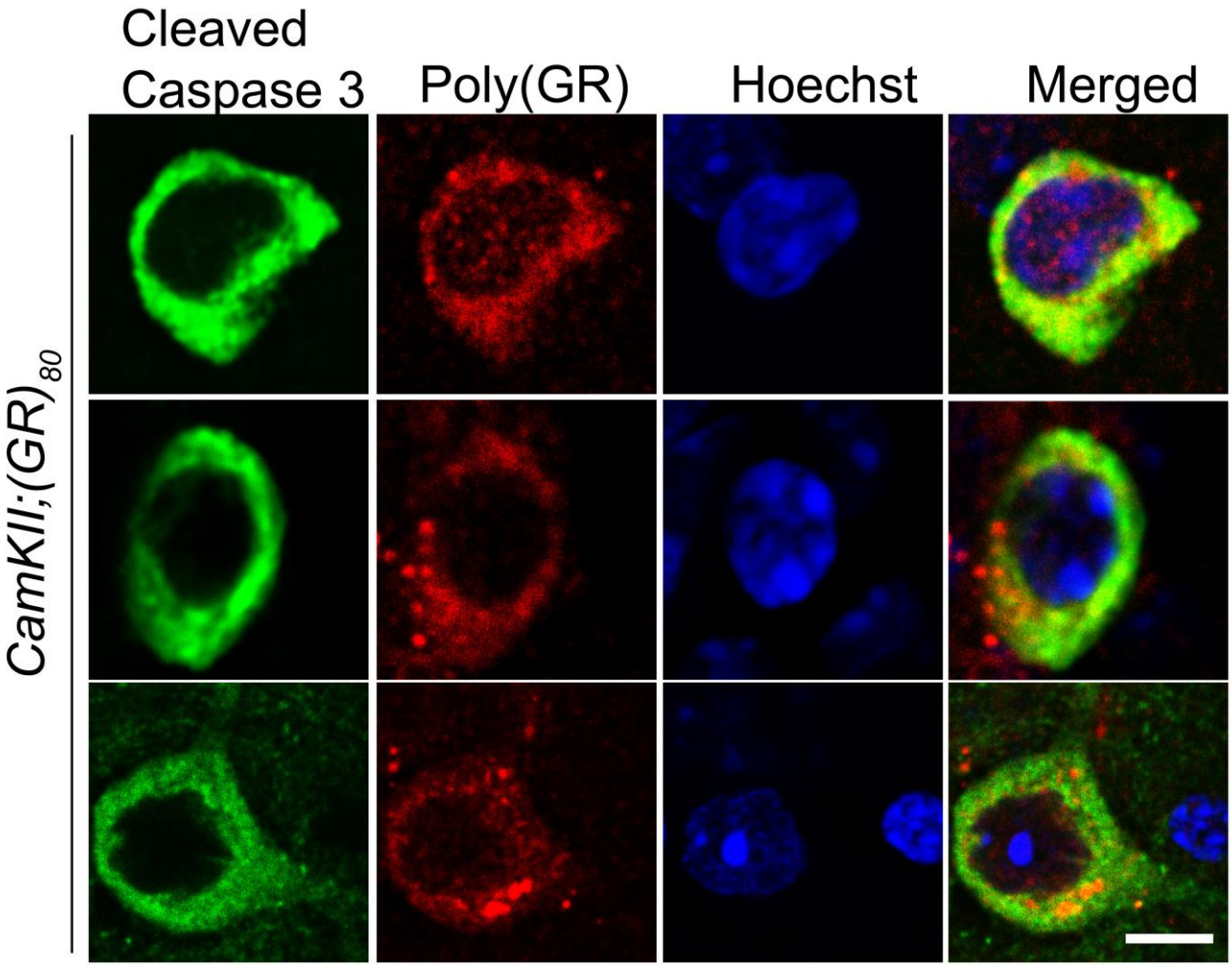
CamKII;(GR)80:female:1 month = 1.75 ± 0.34, n = 6 mice; *CamKII*:female:2 months = 19.00 ± 0.52, n = 6 mice; *CamKII*;(GR)80:female:2 months = 19.00 ± 0.58, n = 6 mice; *CamKII*:female:3 months = 20.00 ± 0.52, n = 6 mice; *CamKII*;(GR)80:female:3 months = 20.17 ± 0.65, n = 6 mice; *CamKII*:female:4 months = 22.17 ± 0.31, n = 6 mice; *CamKII*;(GR)80:female:4 months = 21.50 ± 0.56, n = 6 mice; *CamKII*:female:5 months = 22.83 ± 0.54, n = 6 mice; *CamKII*;(GR)80:female:5 months = 22.50 ± 0.34, n = 6 mice; *CamKII*:female:6 months = 22.83 ± 0.70, n = 6 mice; *CamKII*;(GR)80:female:6 months = 22.83 ± 0.65, n = 6 mice; *CamKII*:female:7 months = 24.00 ± 0.73, n = 6 mice; *CamKII*;(GR)80:female:7 months = 23.83 ± 0.65, n = 6 mice; *CamKII*:female:8 months = 24.67 ± 0.80, n = 6 mice; *CamKII*;(GR)80:female:8 months = 24.33 ± 0.56, n = 6 mice; *CamKII*:female:9 months = 24.50 ± 0.85, n = 6 mice; *CamKII*;(GR)80:female:9 months = 23.83 ± 0.79, n = 6 mice; *CamKII*:female:10 months = 25.17 ± 0.65, n = 6 mice; *CamKII*;(GR)80:female:10 months = 24.83 ± 0.87, n = 6 mice; *CamKII*:female:11 months = 25.50 ± 0.62, n = 6 mice; *CamKII*;(GR)80:female:11 months = 24.67 ± 1.02, n = 6 mice. Values are mean ± s.e.m. by two-way ANOVA with Bonferroni post hoc test: F(10, 110) = 0.1638, *P* > 0.9999 for *CamKII*:male vs. *CamKII*;(GR)80:male in all months, *P* > 0.9999 for *CamKII*:female vs. *CamKII*;(GR)80:female in all age groups. **b–d,** Locomotor activity of *CamKII* and *CamKII;(GR)80* mice in the open field was measured for 10 min at 3 (b), 6 (c), and 9 (d) months of age. The frequency of entries into the chamber center, the time spent there, the total distance and velocity of movement were recorded. Each dot represents one mouse. No statistically significant differences were found. The following values are all mean ± s.e.m. and analyzed by two-sided Student's t test. In the "Frequency in Center" graph in Panel b, *CamKII* = 34.83 ± 4.24 (n = 12 mice), *CamKII*;(GR)80=29.25 ± 2.79 (n = 12 mice), F(1, 22) = 2.315, *P* = 0.2833. In the "Duration in Center" graph Panel b, *CamKII* = 73.36 ± 11.68 (n = 12 mice),; *CamKII;(GR)80* = 83.37 ± 20.06 (n = 12 mice), F(1, 22) = 2.95, *P* = 0.6704. In the "Total Distance" graph in Panel b, *CamKII* = 2510 ± 215.30 (n = 12 mice), *CamKII;(GR)80* = 2390 ± 202.00 (n = 12 mice), F(1, 22) = 1.14, *P* = 0.6704. In the "Velocity" graph in Panel b, *CamKII* = 4.18 ± 0.36 (n = 12 mice), *CamKII;(GR)80* = 3.98 ± 0.34 (n=12 mice), F(1, 22) = 1.14, *P* = 0.69. In the "Frequency in Center" graph Panel c, *CamKII* = 25.36 ± 4.77 (n=11 mice), *CamKII;(GR)80* = 29.56 ± 4.59 (n = 9 mice), F(1, 18) = 1.32, *P* = 0.5404. In the "Duration in Center" graph Panel c, *CamKII* = 42.1 ± 9.47 (n = 9 mice), *CamKII;(GR)80* = 46.05 ± 7.38 (n = 9 mice), F(1, 18) = 2.01, *P* = 0.7541. In the "Total Distance" graph in Panel c, *CamKII* = 2200 ± 231.30 (n=11 mice), *CamKII;(GR)80* = 2457 ± 205.1 (n = 9 mice), F(1, 18) = 1.55, *P* = 0.4267. In the "Velocity" graph in the panel c, *CamKII* = 3.67 ± 0.39 (n = 11 mice), *CamKII;(GR)80* = 4.10 ± 0.34 (n = 9 mice), F(1, 18) = 1.55, *P* = 0.4265. In the "Frequency in Center" graph in Panel d, *CamKII* = 26.80 ± 5.62 (n = 10 mice), *CamKII;(GR)80* = 26.5 ± 3.22 (n = 8 mice), F(1, 16) = 3.81, *P* = 0.9660. In the "Duration in Center" graph in Panel d, *CamKII* = 33.86 ± 9.13, (n = 10 mice); *CamKII;(GR)80* = 39.21 ± 15.11 (n = 8 mice), F(1, 16) = 2.19, *P* = 0.7554. In the "Total Distance" graph in Panel d, *CamKII* = 2596 ± 248.3 (n = 10 mice), *CamKII*;(GR)80= 3035 ± 253.6 (n =8 mice), *P* = 0.2387. In the "Velocity" graph in Panel d, *CamKII* = 4.33 ± 0.41 (n = 10 mice), *CamKII;(GR)80* = 5.06 ± 0.42 (n = 8 mice), F(1, 16) = 1.20, *P* = 0.2387.



Supplementary Figure 4

T-maze working memory test of *CamKII* and *CamKII;(GR)80* mice

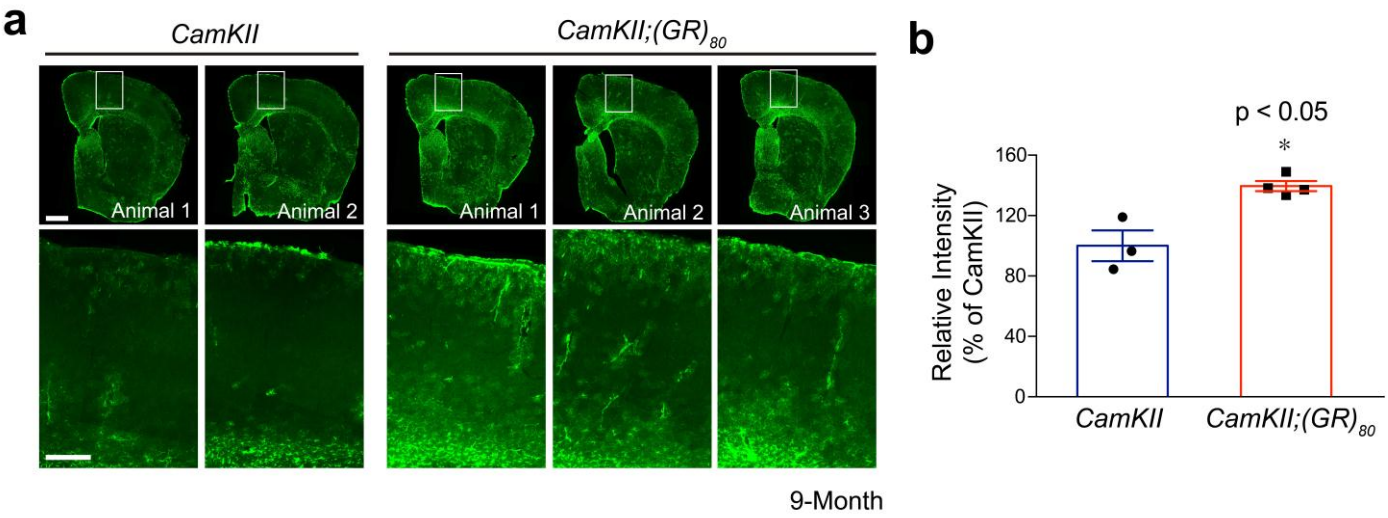
a, Schematic of the T-maze working memory test. The test mouse was forced into the left arm, where food was placed. After a 10-s delay, the mouse was placed in the center arm and allowed to move into the right or left arm. If the mouse chose the arm where the food was, the event was counted as a success. **b**, Success rate of *CamKII* and *CamKII;(GR)80* mice at 6–9 months of age in the T-maze test, calculated from 10 trials. Each dot represents one mouse. No statistically significant difference was found. *CamKII* = 61.11 ± 6.33 (n = 9 mice), *CamKII;(GR)80* = 54.00 ± 4.52 (n = 10 mice). Values are mean ± s.e.m., F(1, 17) = 1.77, *P* = 0.3666, by two-sided Student's t test.



Supplementary Figure 5

Expression of activated caspase-3 in poly(GR)-expressing neurons of three *CamKII;(GR)80* mice

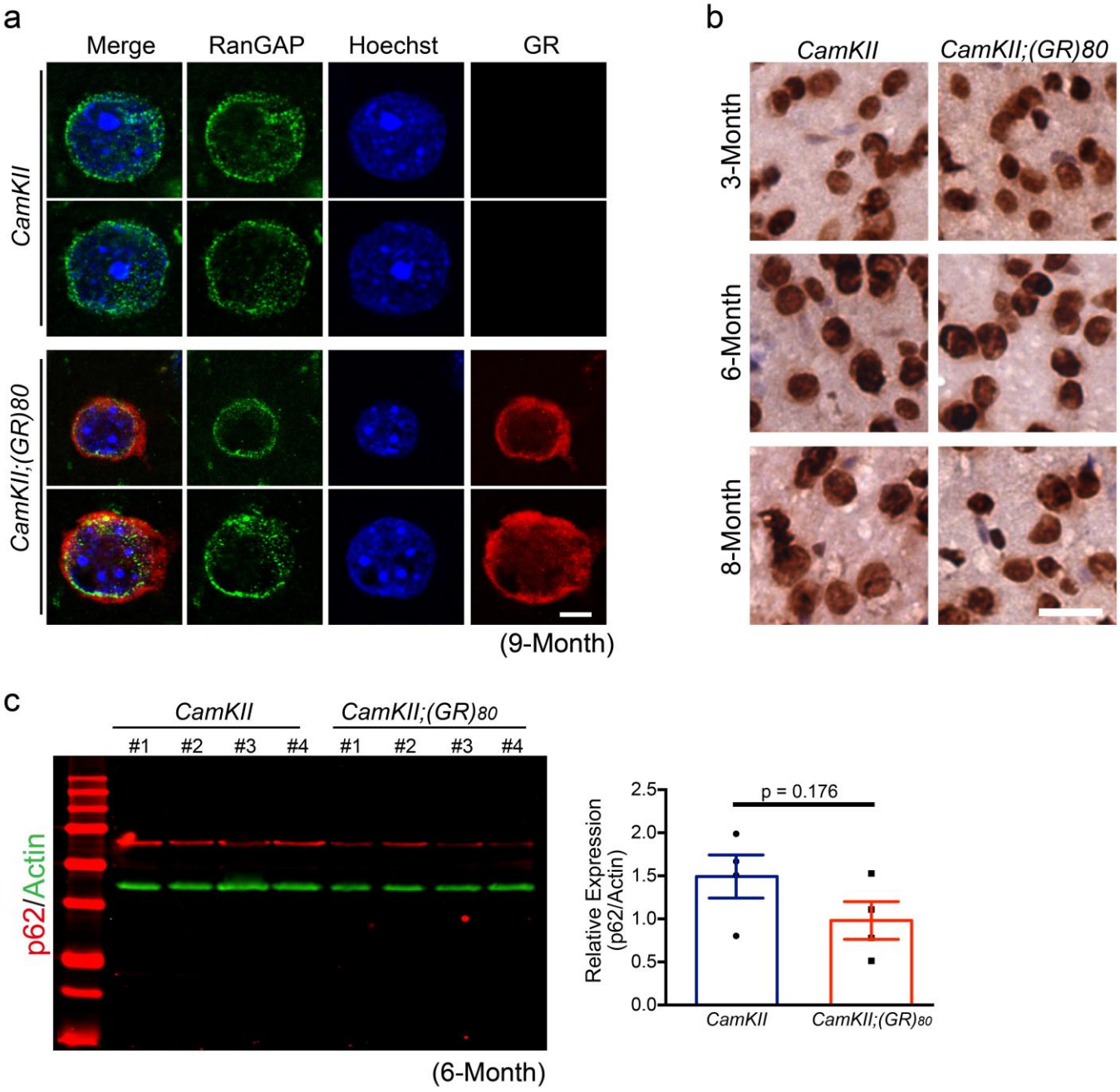
Double-immunostaining for poly(GR) and cleaved caspase 3 in the cortex of three 9-month-old *CamKII;(GR)80* mice. The immunostaining experiments was repeated three times. Scale bar: 10 µm.



Supplementary Figure 6

Astrogliosis in the cortex of *CamKII;(GR)80* mice.

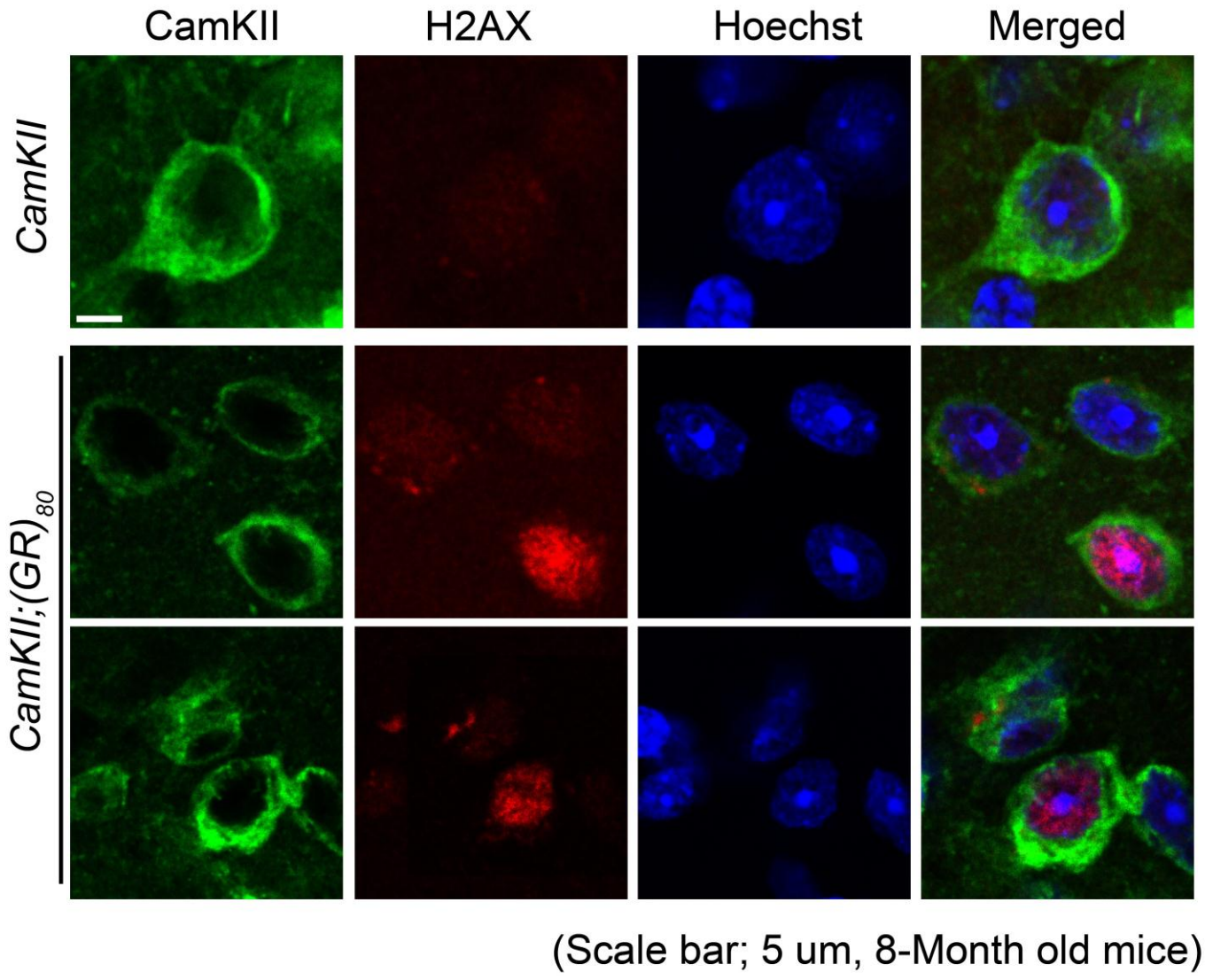
a, Astrogliosis in *CamKII* (n = 2) and *CamKII;(GR)80* (n = 3) mice at 9 months of age as shown by immunostaining for glial fibrillary acidic protein (Gfap). The lower panels are enlarged images of areas indicated by white boxes in corresponding upper panels. Scale bar: 1 mm in upper panels, 300 µm in lower panels. **b,** Quantification of relative intensity of Gfap signal in the cortex of 9-month *CamKII* and *CamKII;(GR)80* mice. *CamKII* = 100 ± 0.08 (n = 3 mice), *CamKII;(GR)80* = 134 ± 0.05 (n = 4 mice). Values are mean ± s.e.m., F(1, 5) = 2.4, *P* = 0.0116, by two-sided Student's t test.



Supplementary Figure 7

Some known molecular defects in *C9ORF72*-FTD/ALS are absent in *CamKII;(GR)80* mice

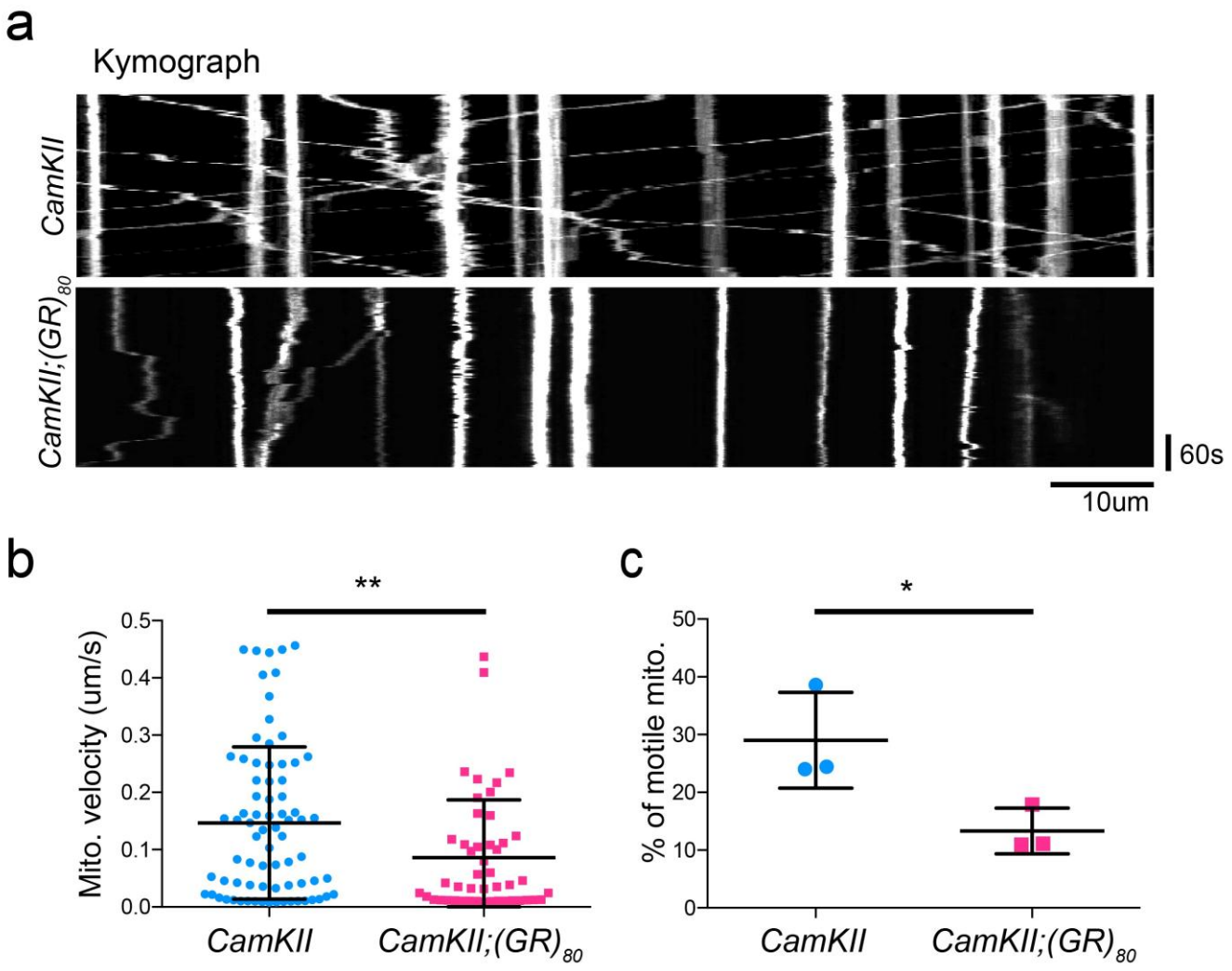
a, Absence of RanGAP1 aggregates in the poly(GR)-expressing neurons of three 9-month-old *CamKII;(GR)80* mice. Three animals per genotype and per age group were analyzed. **b,** Lack of TDP-43 pathology, shown by immunostaining in the cortex of *CamKII* and *CamKII;(GR)80* mice at 3, 6, and 8 months of age. Three animals per genotype and per age group were analyzed. Scale bars: 20 µm. **c,** Western blot analysis and quantification show that the p62 level was not affected in 6-month-old *CamKII;(GR)80* mice. *CamKII* = 1.49 ± 0.25 (n = 4 mice), *CamKII;(GR)80* = 0.98 ± 0.22 (n = 4 mice). Values are mean ± s.e.m., F(1, 6) = 1.31, *P* = 0.1760, by two-sided Student's t test.



Supplementary Figure 8

Increased DNA damage in poly(GR)-expressing neurons of *CamKII;(GR)80* mice

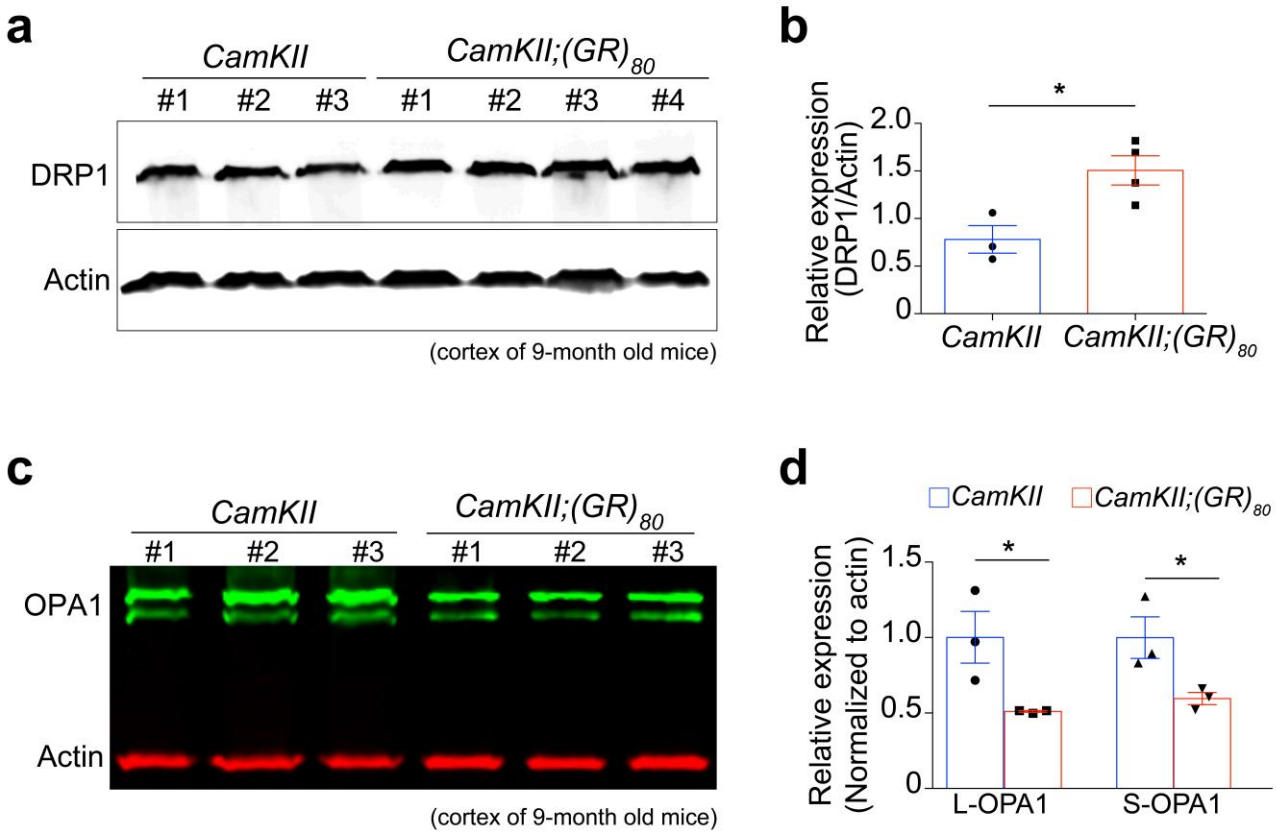
Double-immunostaining for *CamKII* and Histone H2AX (DNA damage marker) in the cortex of three 8-month-old *CamKII;(GR)80* mice. Because (GR)80 expression is driven by *CamKII*-tTA, *CamKII*-positive neurons express (GR)80. Scale bar: 5 µm.



Supplementary Figure 9

Reduced mitochondria motility in cultured primary neurons of *CamKII;(GR)80* mice

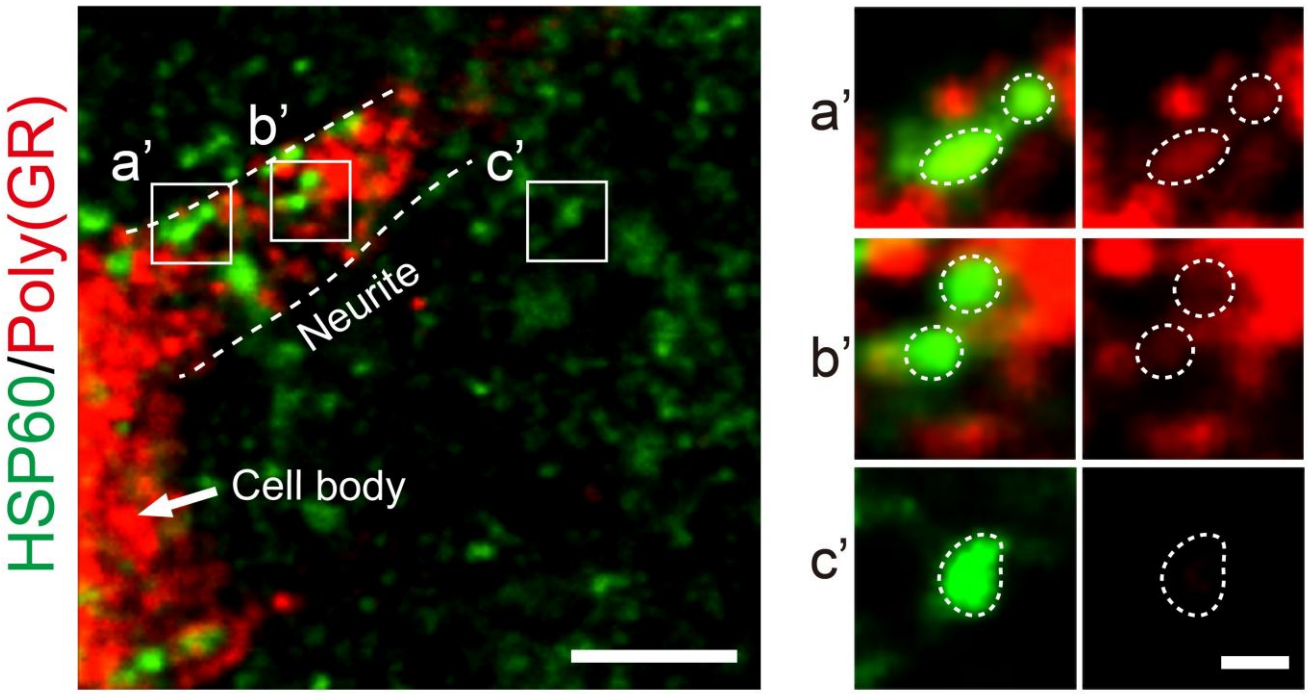
a, A representative kymograph of mitochondrial movement in neurites of *CamKII* and *CamKII;(GR)80* primary cortical neurons on day 14 in vitro (DIV14). The experiment was performed in three independent cultures from three different animals. Scale bar: 10 µm. **b,** The velocity of mitochondria in neurite of *CamKII* and *CamKII;(GR)80* primary cortical neurons on DIV14. Each dot represents one mitochondrion. *CamKII* = 0.15 ± 0.01 (n=76 mitochondria), *CamKII;(GR)80* = 0.09 ± 0.01 (n = 47 mitochondria). Values are means ± s.d., F(1, 121) = 1.74, *P* = 0.0085, by two-sided Student's t test. **c.** The percentage of mobile mitochondria in poly(GR)-expressing primary cortical neurons on DIV14. The primary cortical neurons were cultured from three embryos of either *CamKII* or *CamKII;(GR)80* mice, and mitochondrial movement was analyzed on DIV14. *CamKII* = 29.01 ± 4.80 (n = 3 mice), *CamKII;(GR)80* = 13.32 ± 2.29 (n = 3 mice). Values are mean ± s.e.m., F(1, 4) = 4.39, *P* = 0.0418, by two-sided Student's t test.



Supplementary Figure 10

Changes in DRP1 and OPA1 levels in neurons of *CamKII;(GR)80* mice

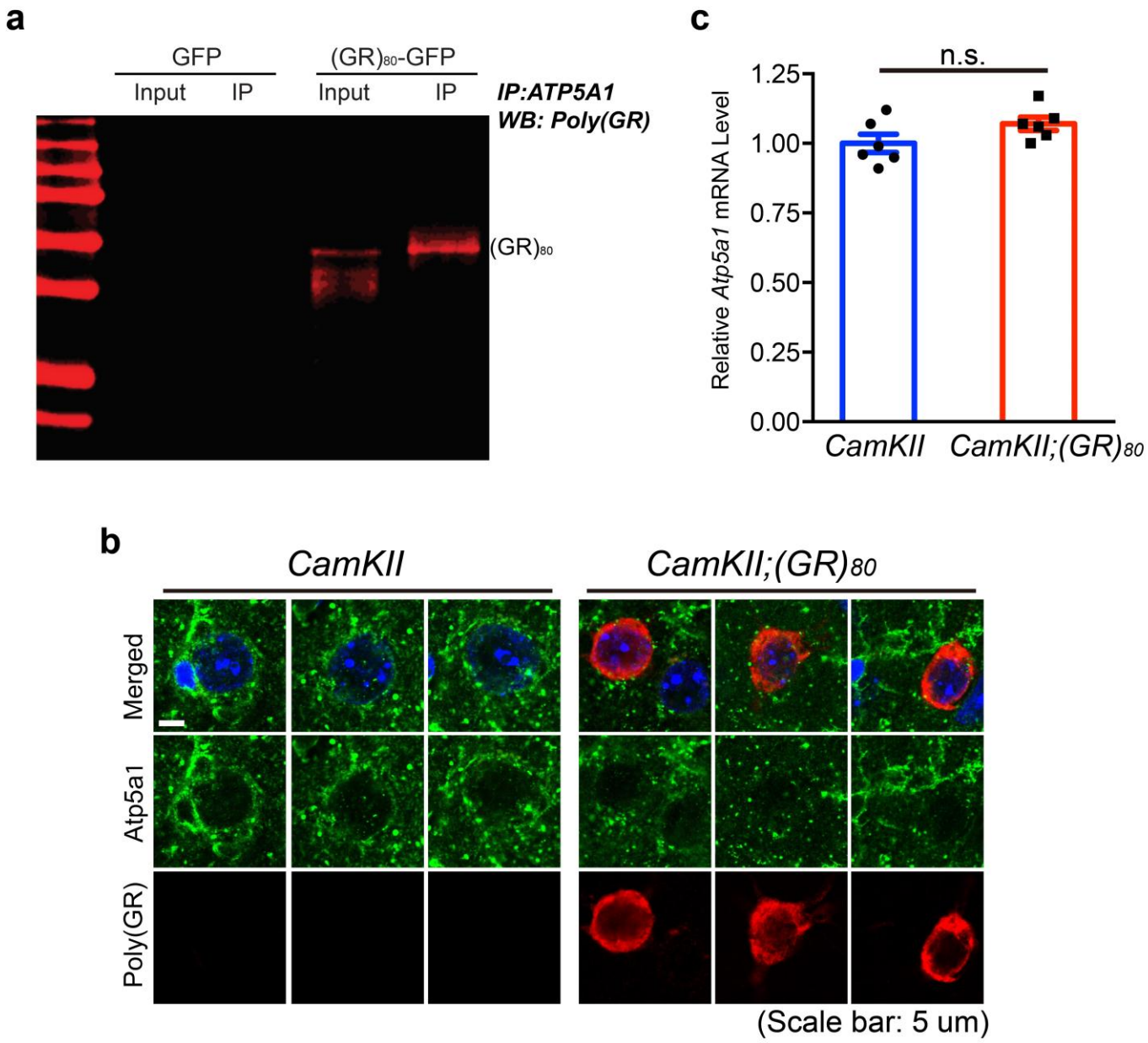
a, Western blot analysis of DRP1 in the cortex of *CamKII* mice (n = 3) and *CamKII;(GR)80* mice (n = 4) at 9 months of age. **b,** Quantification of western blot analysis of DRP1 in the cortex of 9-month old *CamKII* and *CamKII;(GR)80* mice. *CamKII* = 0.78 ± 0.15 (n = 3 mice), *CamKII;(GR)80* = 1.51 ± 0.51 (n = 4 mice). Values are mean ± s.e.m., F(1, 5) = 1.49, *P* = 0.0209, by two-sided Student's t test. **c,** Western blot analysis of OPA1 in the cortex of *CamKII* and *CamKII;(GR)80* mice at 9 months of age. d, Quantification of western blot analysis of long and short OPA1 (L-OPA1, S-OPA1) in the cortex of 9-month-old *CamKII* and *CamKII;(GR)80* mice. *CamKII*:L-OPA1 = 1.00 ± 0.17 (n = 3 mice), *CamKII*;(GR)80:L-OPA1 = 0.51 ± 0.01 (n = 3 mice), *CamKII*:S-OPA1 = 1.00 ± 0.14 (n = 3 mice), *CamKII*;(GR)80:S-OPA1= 0.60 ± 0.04 (n = 3 mice). Values are mean ± s.e.m., F(1, 4) = 880.7, *P* = 0.0467 for *CamKII*:L-OPA1 vs. *CamKII*;(GR)80:L-OPA1, F(1, 4) = 11.61, *P* = 0.0483 for *CamKII*:S-OPA1 vs. *CamKII*;(GR)80:S-OPA1, by two-sided Student's t test.



Supplementary Figure 11

Poly(GR) is present inside mitochondria

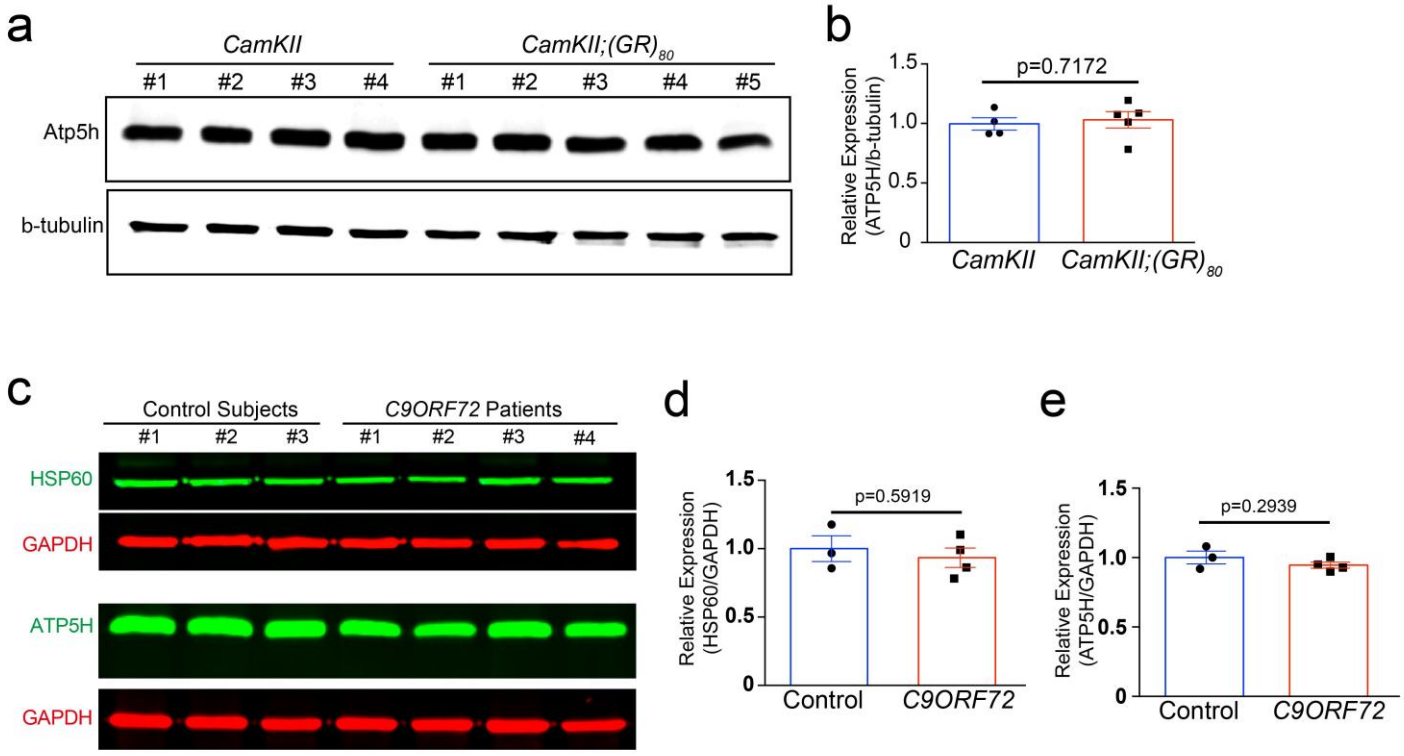
Double-immunostaining for HSP60, a mitochondria-specific marker, and poly(GR) in type C poly(GR)-expressing neurons of two 9-month-old *CamKII;(GR)80* mice with high-level of poly(GR) expression (line 8). This immunostaining experiment was repeated three times. Scale bar: 5 µm. Enlarged squares a', b' and c' are shown on the right. Dotted circles indicate the mitochondrial location (from three independently repeated experiments with similar results). Scale bar: 0.5 µm.



Supplementary Figure 12

Binding of poly(GR) to ATP5A1 and the level of Atp5a1 expression in *CamKII;(GR)80* mice

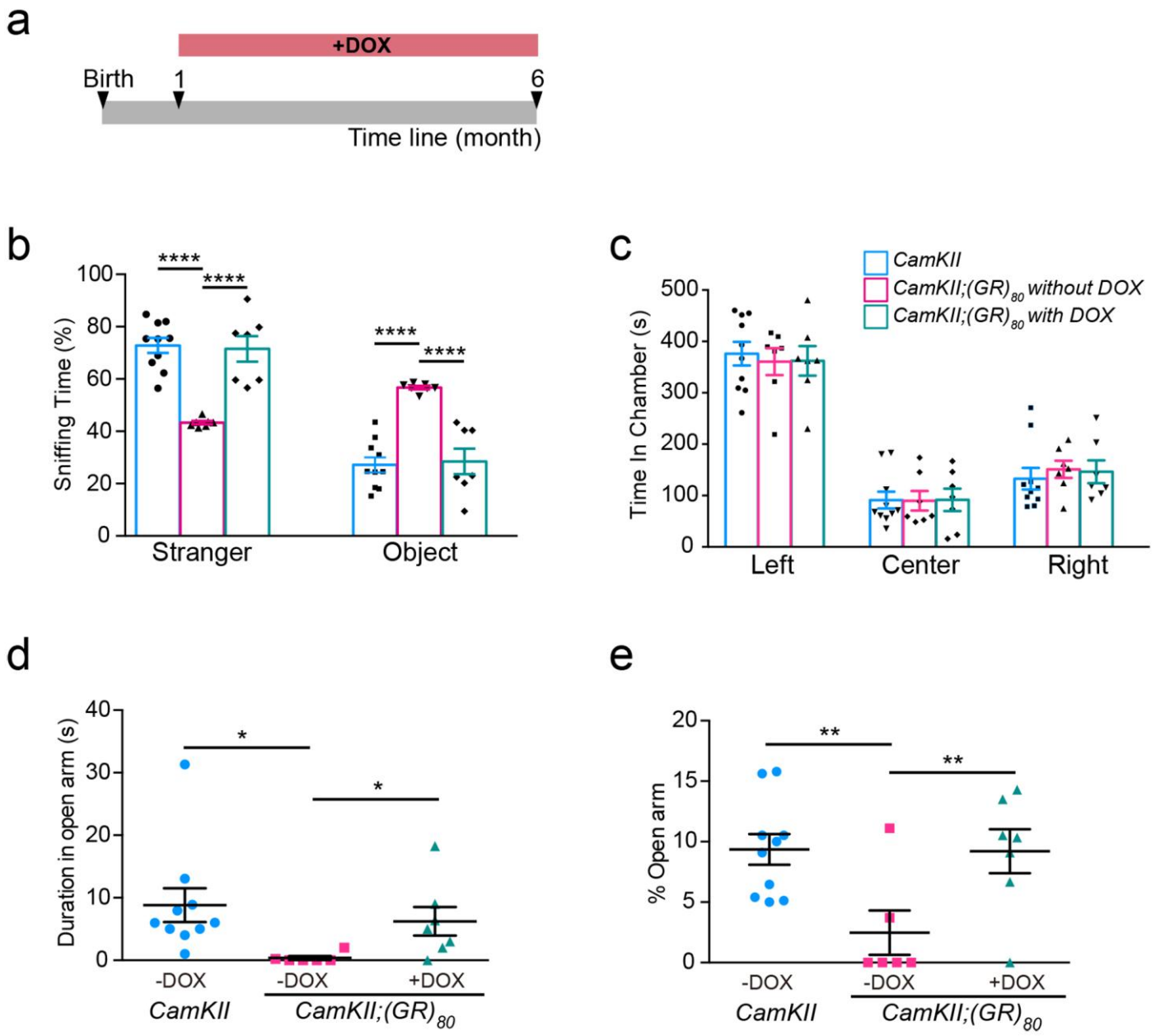
a, Poly(GR) co-immunoprecipitated with ATP5A1 GFP-(GR)80 overexpressing HEK293 cells was analyzed on western blots (from three independently repeated experiments with similar results). **b,** Immunostaining for Atp5a1 in cortical neurons expressing poly(GR) (from three independently repeated experiments with similar results). Scale bar: 5 µm. **c,** Relative expression levels of *Atp5a1* mRNA in *CamKII* and *CamKII;(GR)80* mice. *CamKII* = 1.00 ± 0.03 (n = 6 mice), *CamKII;(GR)80* = 1.10 ± 0.02 (n = 6 mice). Values are mean ± s.e.m., F(1, 10) = 1.86, *P* = 0.1126, by two-sided Student's t test.



Supplementary Figure 13

The expression of some mitochondrial proteins in cortex of *CamKII;(GR)80* mice and *C9ORF72* patients

a, The western blot analysis of Atp5h in the cortex of *CamKII* and *CamKII;(GR)80* mice at 6-month old of age (from three independently repeated experiments with similar results). **b,** Quantification of western blot analysis of Atp5h in the cortex of *CamKII* and *CamKII;(GR)80* mice at 6-month old of age. *CamKII* = 1.00 ± 0.05 (n = 4 mice), *CamKII;(GR)80* = 1.03 ± 0.07 (n = 5 mice). Values are mean ± s.e.m., F(1, 7) = 2.16, *P* = 0.7172, by two-sided Student's t test. **c,** Western blot analysis of HSP60 and ATP5H in the prefrontal cortex of *C9ORF72* patients (from three independently repeated experiments with similar results). **d and e** Quantification of western blot in panel c. Values are means ± s.e.m. In the panel d, Control = 1.00 ± 0.10 (n = 3 subjects), *C9ORF72* = 0.93 ± 0.07 (n = 4 patients), F(1, 5) = 1.33, *P* = 0.5919 by two-sided Student's t test. In the panel e, Control = 1.00 ± 0.05 (n = 3 subjects), *C9ORF72* = 0.95 ± 0.22 (n = 4 patients), F(1, 5) = 3.27, *P* = 0.2939 by two-sided Student's t test.

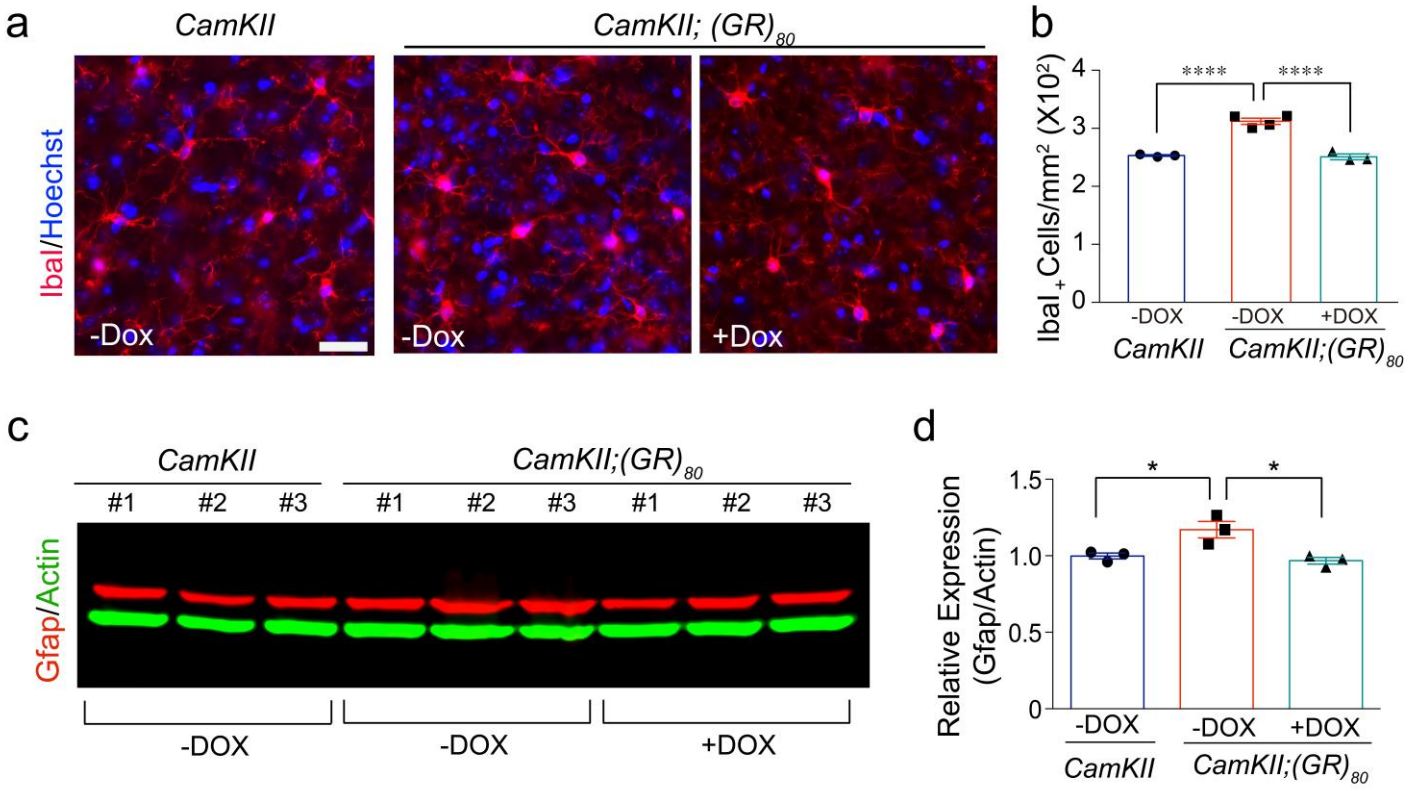


Supplementary Figure 14

Suppression of poly(GR) expression prevents behavioral defects of *CamKII;(GR)80* mice

a, Schematic of doxycycline (DOX) treatment of *CamKII;(GR)80* mice from 1–6 months of age. **b and c,** Results of the three-chamber social interaction test for *CamKII* and *CamKII;(GR)80* mice at 6 months of age. n = 6–10 mice of each genotype at each time point. Each dot represents one mouse. The following values are mean ± s.e.m. and analyzed by two-sided Student's t test: F(2, 24) = 83.9, *P* < 0.0001 for *CamKII*:Stranger vs. *CamKII;(GR)80* without DOX:Stranger, F(2, 24) = 83.9, *P* < 0.0001 for *CamKII;(GR)80* without DOX:Stranger vs. *CamKII;(GR)80* with DOX:Stranger, F(2, 24) = 83.9, *P* < 0.0001 for *CamKII*:Object vs. *CamKII;(GR)80* without DOX:Object, F(2, 24) = 83.9, *P* <0.0001 for *CamKII;(GR)80* without DOX:Object vs. *CamKII;(GR)80* with DOX:Object. In Panel b, *CamKII*:Stranger = 72.83 ± 2.89 (n = 10 mice), *CamKII;(GR)80* without DOX:Stranger = 43.27 ± 0.54 (n = 7 mice), *CamKII;(GR)80* with DOX:Stranger = 71.53 ± 4.08 (n = 7 mice), *CamKII*:Object = 27.17 ± 2.89 (n = 10 mice), *CamKII;(GR)80* without DOX:Object = 56.73 ± 0.54 (n=7 mice), *CamKII;(GR)80* with DOX:Object = 28.47 ± 4.08 (n = 7 mice). In Panel c, *CamKII*:Left = 376.10 ± 22.99 (n = 10 mice), *CamKII;(GR)80* without DOX:Left = 359.29 ± 21.99 (n = 7 mice), *CamKII;(GR)80* with DOX:Left = 362.14 ± 24.11 (n = 7 mice), *CamKII*:Center = 91.15 ± 16.28 (n = 10 mice), *CamKII;(GR)80* without DOX:Center = 89.75 ± 15.84 (n = 7 mice), *CamKII;(GR)80* with

DOX: Center = 91.65 ± 18.25 (n = 7 mice), *CamKII*:Right = 132.75 ± 21.00 (n = 10 mice), *CamKII;(GR)80* without DOX:Right = 150.96 ± 13.9 (n = 7 mice), *CamKII;(GR)80* with DOX:Right = 146.21 ± 18.62 (n = 7 mice). **d and e**, Duration (d) and percentage of entries (e) in the open arm of the elevated plus maze by *CamKII* and *CamKII;(GR)80* mice at 6 months of age. In Panel d, *CamKII* = 8.82 ± 2.70 (n = 10 mice), *CamKII;(GR)80* without DOX = 0.37 ± 0.33 (n = 6 mice), *CamKII;(GR)80* with DOX = 6.23 ± 2.30 (n = 6 mice), F(1, 14) = 112.5, *P* = 0.0317 for *CamKII* vs. *CamKII;(GR)80* without DOX, F(1, 11) = 83.9, *P* = 0.0394 for *CamKII;(GR)80* without DOX vs. *CamKII;(GR)80* with DOX, by two-sided Student's t test. In Panel e, *CamKII* = 9.35 ± 1.27 (n = 10 mice), *CamKII;(GR)80* without DOX = 2.47 ± 1.83 (n= 6 mice), *CamKII;(GR)80* with DOX = 9.20 ± 1.82 (n = 7 mice), F(1, 14) = 1.25, *P* = 0.0066 for *CamKII* vs. *CamKII;(GR)80* without DOX, F(1, 11) = 1.15, *P* = 0.0249 for *CamKII;(GR)80* without DOX vs. *CamKII;(GR)80* with DOX, by two-sided Student's t test.



Supplementary Figure 15

Feeding doxycycline for two months reverses increased microgliosis and astrogliosis in 9-month-old *CamKII;(GR)80* mice

a, Representative images of Iba1-positive cells in the cortex of three *CamKII* and *CamKII;(GR)80* mice of 9-month-old. Scale bar: 25 μm. **b,** Quantification of Iba1-positive cells in the cortex of 9-month-old *CamKII* and *CamKII;(GR)80* mice. *CamKII* = 253.09 ± 1.28 (n =3 mice), *CamKII;(GR)80* without DOX = 312.22 ± 5.23 (n = 4 mice), *CamKII;(GR)80* with DOX = 250.88 ± 4.98 (n = 3 mice). Values are mean ± s.e.m., $F(2, 7) = 1.96$, $P < 0.0001$ for *CamKII* vs. *CamKII;(GR)80* without DOX, $P < 0.0001$ for *CamKII;(GR)80* without DOX vs. *CamKII;(GR)80* with DOX, by one-way ANOVA with Tukey's post hoc analysis for multiple comparisons. **c,** Western blot analysis of Gfap expression in the cortex of *CamKII* and *CamKII;(GR)80* mice fed normal chow or chow containing doxycycline (DOX). Three mice of each genotype and treatment were analyzed (from three independently repeated experiments with similar results). **d,** Quantification of Gfap expression level. *CamKII* = 1.00 ± 0.02 (n = 3 mice) *CamKII;(GR)80* without DOX = 1.17 ± 0.05 (n = 3 mice), *CamKII;(GR)80* with DOX = 0.97 ± 0.02. Values are mean ± s.e.m., $F(2, 6) = 9.68$, $P = 0.0311$ for *CamKII* vs. *CamKII;(GR)80* without DOX, $P = 0.0153$ for *CamKII;(GR)80* without DOX vs. *CamKII;(GR)80* with DOX, by two-sided Student's t test.

Table S1. Clinical Information for Human Brain Tissues Used in This Study.

Brain number	**Sex**	**Age at death**	**Clinical diagnosis**	**Primary Neuropathological diagnosis**	**Post-mortem Interval (hrs)**	**Anatomical region (gyrus)**
C9orf72-1	M	67	bvFTD	FTLD-TDP-B	1.7	Superior frontal gyrus, anterior
C9orf72-2	M	48	bvFTD-MND	FTLD-TDP-B	21.0	Superior frontal gyrus, anterior
C9orf72-3	F	60	bvFTD-MND	FTLD-TDP-B, MND	11.9	Superior frontal gyrus, anterior
C9orf72-4	F	64	bvFTD	FTLD-TDP-B, MND	24.3	Superior frontal gyrus, anterior
Control-1	F	86	Control	Cerebrovascular disease, AGD	7.8	Superior frontal gyrus, anterior
Control-2	M	76	Control	None	8.2	Superior frontal gyrus, anterior
Control-3	F	86	Control	iLBD, brainstem predominant	6.4	Superior frontal gyrus, anterior