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Supplementary Data

# Parkin Null Cortical Neuronal/Glial Cultures are Resistant to Amyloid-$\beta_{1\text{-}42}$ Toxicity: A Role for Autophagy?

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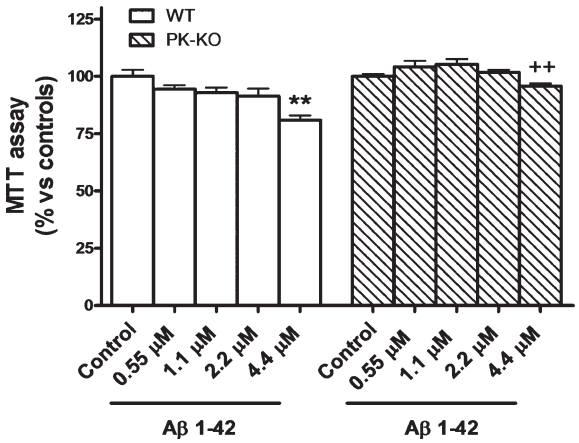


Supplementary Figure 1. Dose-response curve effects of $A\beta_{1\text{-}42}$ in WT and PK-KO cortical cultures. After 5 days *in vitro* (DIV), WT and PK-KO neuron-glia cultures were treated with oligomeric $A\beta_{1\text{-}42}$ (0; 0.55; 1.1; 2.2 or 4.4 μM) for 48 h. Mitochondrial activity was measured by MTT assay. Values are the mean ± SEM from one experiment with 6 replicates. Statistical analysis was performed by one-way ANOVA followed by Newman Keuls multiple comparison test. $^{**}p < 0.01$ Aβ-treated cultures versus their respective controls; $^{++}p < 0.01$ PK-KO versus WT cultures.

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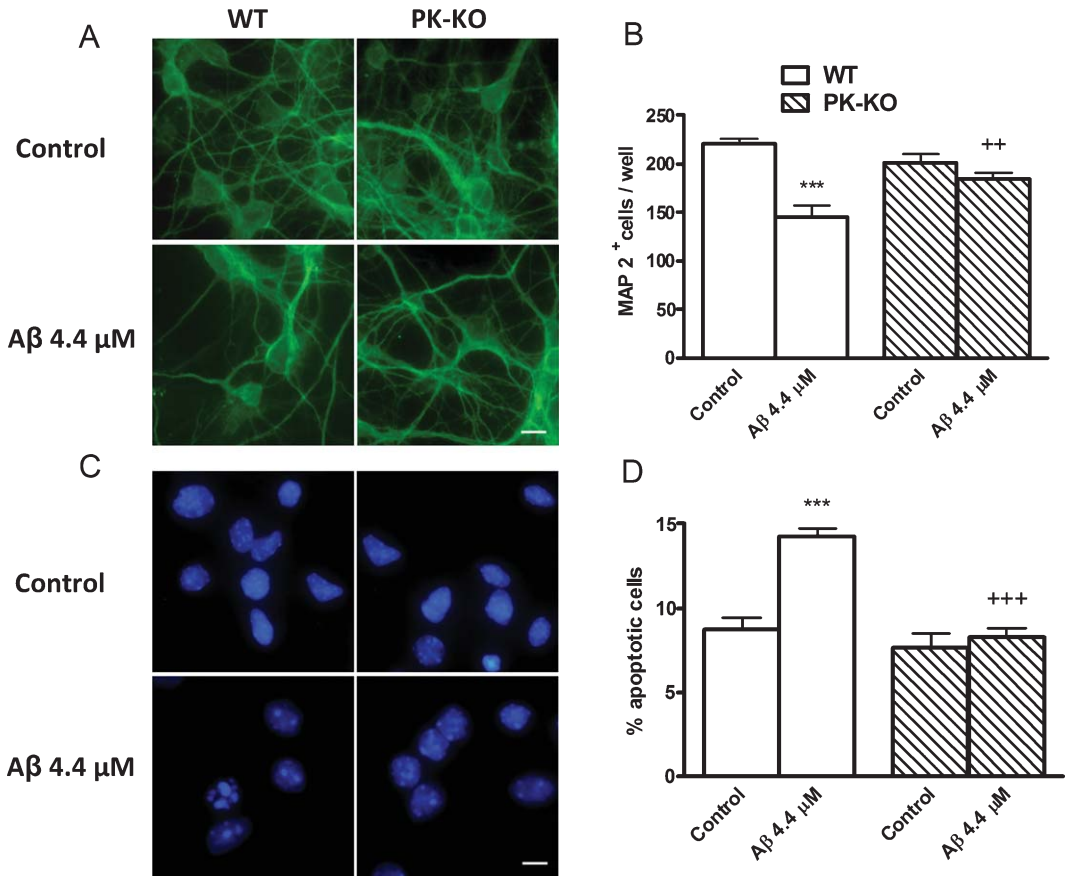
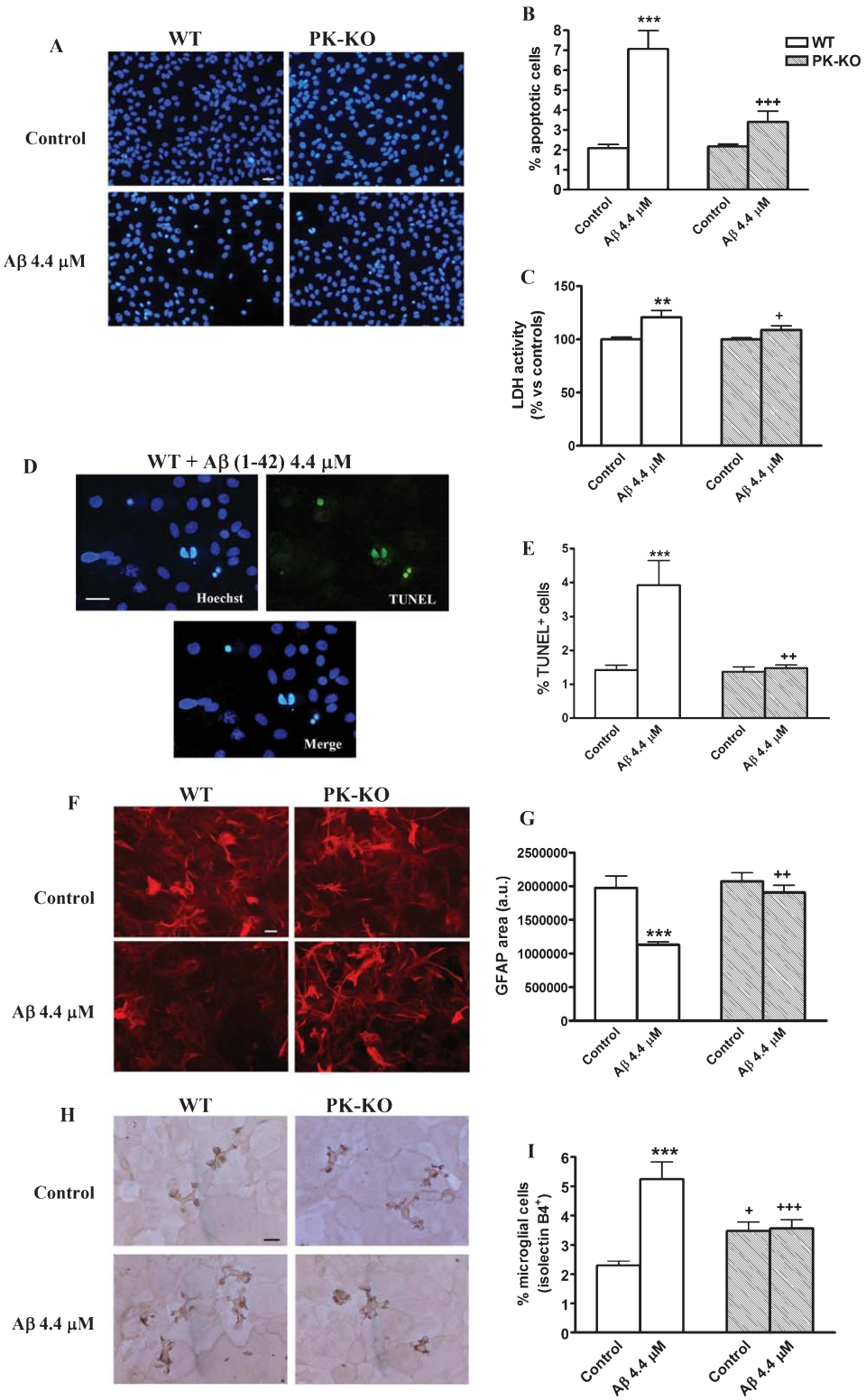
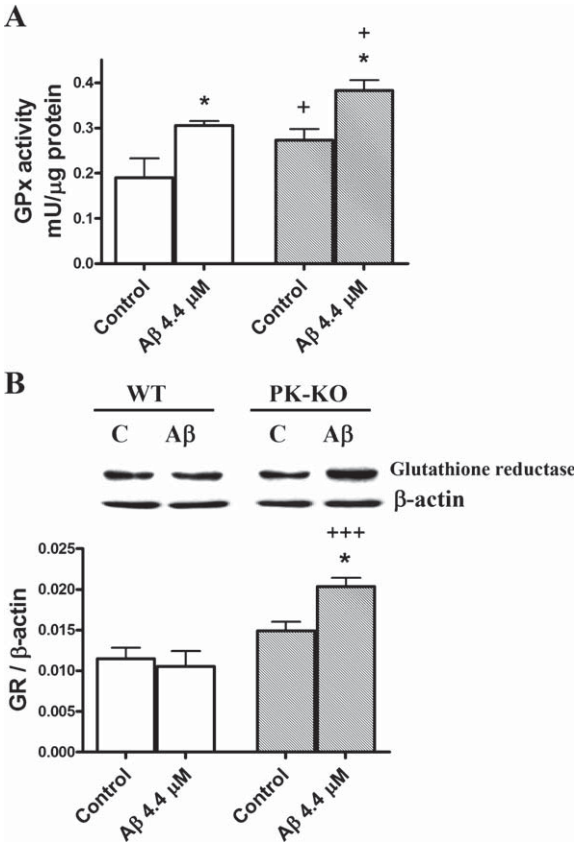



Supplementary Figure 2. Differential effects of $A\beta_{1\text{-}42}$ on cell death in WT and PK-KO neuronal-enriched cultures. PK-KO cultures are resistant to cell death induced by oligomeric form of $A\beta_{1\text{-}42}$. Twenty-four hours after plating, the cells were changed to serum-free defined medium and after 5 DIV, the cultures were treated with $A\beta_{1\text{-}42}$ at 4.4 μM for 48 h. A) Photomicrographs and (B) quantification of total neurons ($MAP2^+$ cells) in WT and PK-KO cultures from control and $A\beta_{1\text{-}42}$ treated cells. C) Photomicrographs of total nuclei stained with bis-benzimide. D) Percentage of apoptotic cells with respect to the total cell number in WT and PK-KO neuronal cultures treated with $A\beta_{1\text{-}42}$ or solvent. Scale bar, 10 μm. Values are the mean ± SEM from two independent experiments with 4–6 replicates each. Statistical analysis was performed by one or two-way ANOVA followed by Newman Keuls multiple comparison test and Bonferroni post-test, respectively. $^{***}p < 0.001$ Aβ treated cultures versus their respective controls; $^{++}p < 0.05$, $^{+++}p < 0.001$ PK-KO versus WT cultures. The interaction between the genotype and the treatment was $F(1,16) = 0.002$; $p < 0.0001$ for D.

Supplementary Figure 3. Differential effects of $A\beta_{1\text{-}42}$ on cell death and glial phenotypes in WT and PK-KO glial cultures. WT and PK-KO cortical glial cultures of 20–30 DIV growing in DEMEM plus 15% FCS (growth medium) were used; 6-7 days after reseeding, glial cultures were incubated in EF12 medium (defined medium) with $A\beta_{1\text{-}42}$ (4.4 μM for 48 h) or solvent. A) Photomicrographs of total nuclei stained with bis-benzimide. B) Percentage of apoptotic cells with respect to the total cell number in WT and PK-KO glial cultures treated with $A\beta_{1\text{-}42}$ or solvent. C) LDH activity. D) Photomicrographs of total nuclei stained with bis-benzimide and positive co-localization of apoptotic cells obtained by the TUNEL assay corresponding to the same field in WT glial cultures. E) Percentage of $TUNEL^+$cells in WT and PK-KO glial cultures. F) Photomicrographs showing type 2 astrocytes ($GFAP^+$ cells) in WT and PK-KO from control and $A\beta_{1\text{-}42}$ treated cells. G) Astroglial immunoreactivity (GFAP) quantification in the cultures. Photomicrographs (H) and percentage of microglial cells (I) (isolectin $B4^+$ cells) in WT and PK-KO cultures treated with $A\beta_{1\text{-}42}$ or solvent. Scale bar, 30 μm. Values are the mean ± SEM from two independent experiments with 6 replicates each. Statistical analysis was performed by one or two-way ANOVA followed by Newman Keuls multiple comparison test and Bonferroni post-test, respectively. $^{**}p < 0.01$, $^{***}p < 0.001$ Aβ-treated cultures versus their respective controls; $^{+}p < 0.05$, $^{++}p < 0.01$, $^{+++}p < 0.001$ PK-KO versus WT cultures. The interaction between the genotype and the treatment was $F(1,60) = 12.73$; $p = 0.0007$ for B. $F(1,14) = 12.06$; $p = 0.0037$ for E. $F(1,32) = 7.15$; $p = 0.011$ for G and $F(1,47) = 13.0$; $p = 0.0007$ for I.




Supplementary Figure 3.




Supplementary Figure 4. Glutathione peroxidase activity and glutathione reductase protein levels in WT and PK-KO, control, and Aβ treated cultures. A) Specific activity of GPx enzyme in neuronal/glia mixed cultures. The activity of the detoxification enzyme was expressed as mU/μ gr protein. B) Western blot of glutathione reductase protein corrected by β-actin expression in WT and PK-KO neuronal/glial cultures under Aβ treatment. Values are the mean SEM of two or three independent cultures with six replicates each. Statistical analysis was performed by one-way ANOVA, followed by Newman–Keuls multiple comparison test. $^{*}p < 0.05$ Aβ treated-cultures versus their respective controls; $^{+}p < 0.05$; $^{+++}p < 0.001$ PK-KO versus WT cultures.

Supplementary Table 1
Cellular characterization of fetal cortical neuron-enriched and neuron-glia mixed cultures from WT and PK-KO mice

| | Neuronal-enriched cortical cultures | | Neuron/glia mixed cortical cultures | |
|---|---|---|---|---|
| | WT | PK-KO | WT | PK-KO |
| % Neuron cells ($MAP2^+$) | $74.32 \pm 3.25\%$ | $81.36 \pm 3.58\%$ | $48.1 \pm 2.66\%$ | $59.47 \pm 3.47\%$ |
| % Type 2 Astrocyte cells ($GFAP^+$) | $10.46 \pm 0.79\%$ | $11.73 \pm 1.13\%$ | $25 \pm 1.23\%$ | $25.96 \pm 1.3\%$ |
| % Microglial cells (Isolectine $B4^+$) | $1.57 \pm 0.12\%$ | $3.37 \pm 0.5\%^{+++}$ | $2.1 \pm 0.12\%$ | $3.06 \pm 0.21\%^{++}$ |

The percentages of the different neuronal and glial cell types from WT and PK-KO cultures are expressed as the mean ± SEM from six replicates of two to four independent experiments. Statistical analysis was performed by one-way ANOVA followed by Newman Keuls multiple comparison test. $^{++}p < 0.005$; $^{+++}p < 0.001$ PK-KO versus WT.