

Supplementary Data

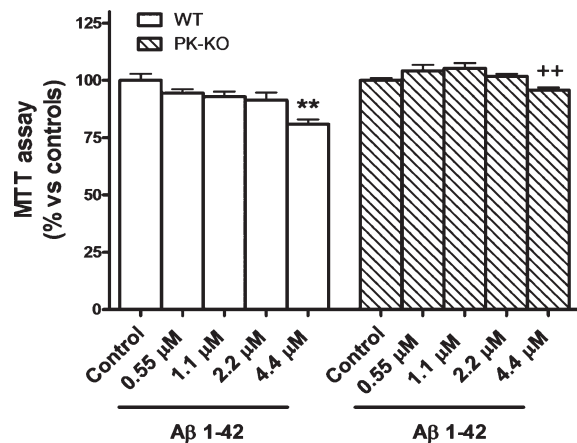
Parkin Null Cortical Neuronal/Glial Cultures are Resistant to Amyloid- β_{1-42} Toxicity: A Role for Autophagy?

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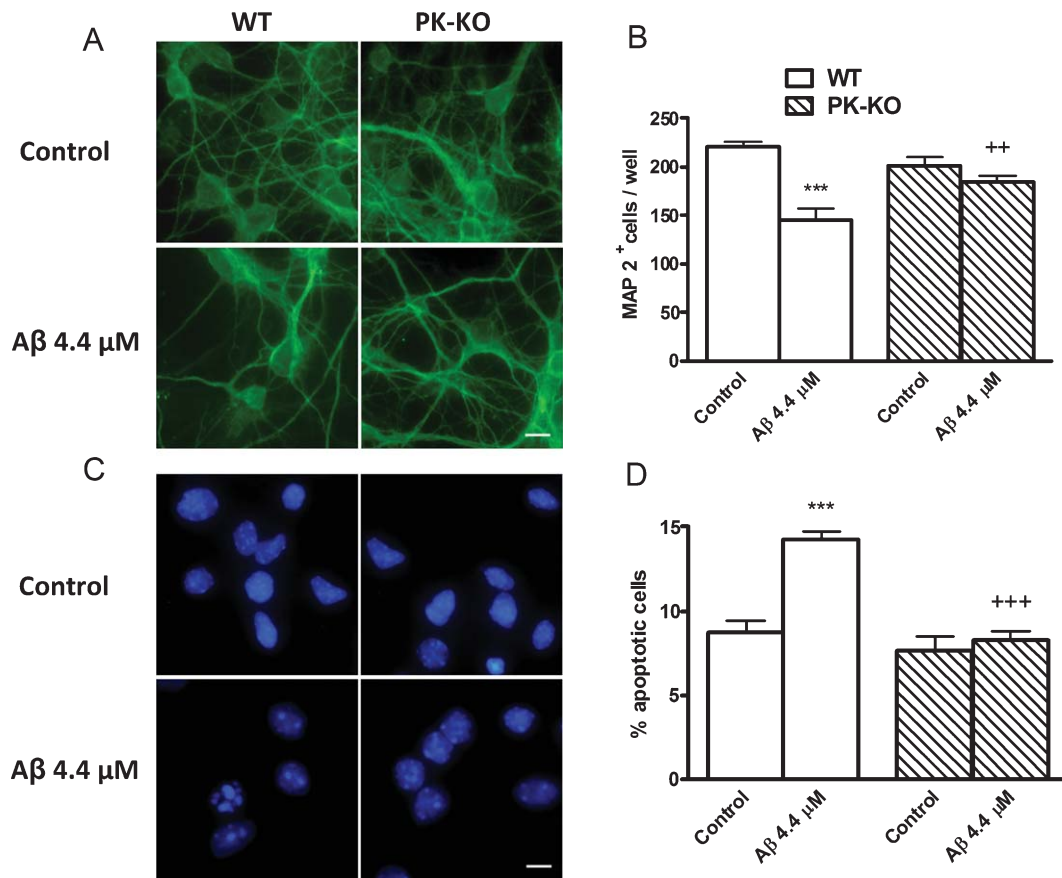
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Supplementary Figure 1. Dose-response curve effects of $\text{A}\beta_{1-42}$ in WT and PK-KO cortical cultures. After 5 days *in vitro* (DIV), WT and PK-KO neuron-glia cultures were treated with oligomeric $\text{A}\beta_{1-42}$ (0; 0.55; 1.1; 2.2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

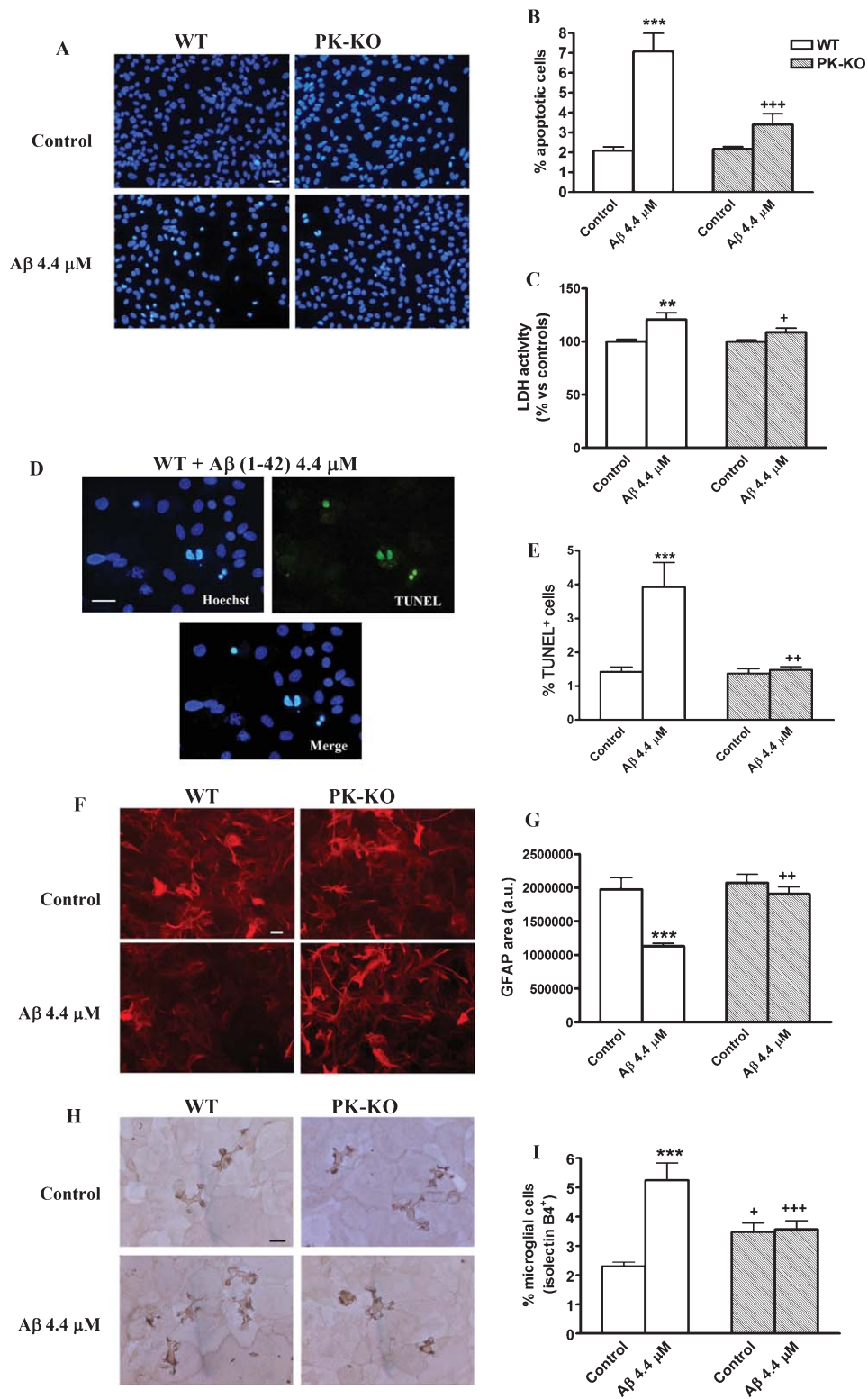
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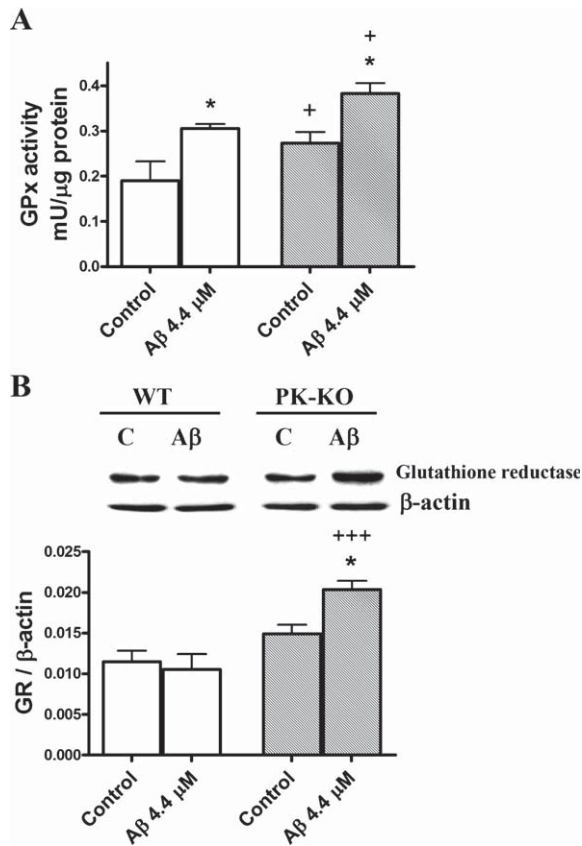


Supplementary Figure 2. Differential effects of A β ₁₋₄₂ on cell death in WT and PK-KO neuronal-enriched cultures. PK-KO cultures are resistant to cell death induced by oligomeric form of A β ₁₋₄₂. Twenty-four hours after plating, the cells were changed to serum-free defined medium and after 5 DIV, the cultures were treated with A β ₁₋₄₂ at 4.4 μ M for 48 h. A) Photomicrographs and (B) quantification of total neurons (MAP2⁺ cells) in WT and PK-KO cultures from control and A β ₁₋₄₂ 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 β ₁₋₄₂ or solvent. Scale bar, 10 μ m. Values are the mean \pm 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 A β 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 β ₁₋₄₂ 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 β ₁₋₄₂ (4.4 μ M 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 β ₁₋₄₂ 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 β ₁₋₄₂ 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 β ₁₋₄₂ or solvent. Scale bar, 30 μ m. Values are the mean \pm 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 A β -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 A β 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/glia cultures under A β 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. * $p < 0.05$ A β 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 \pm 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.