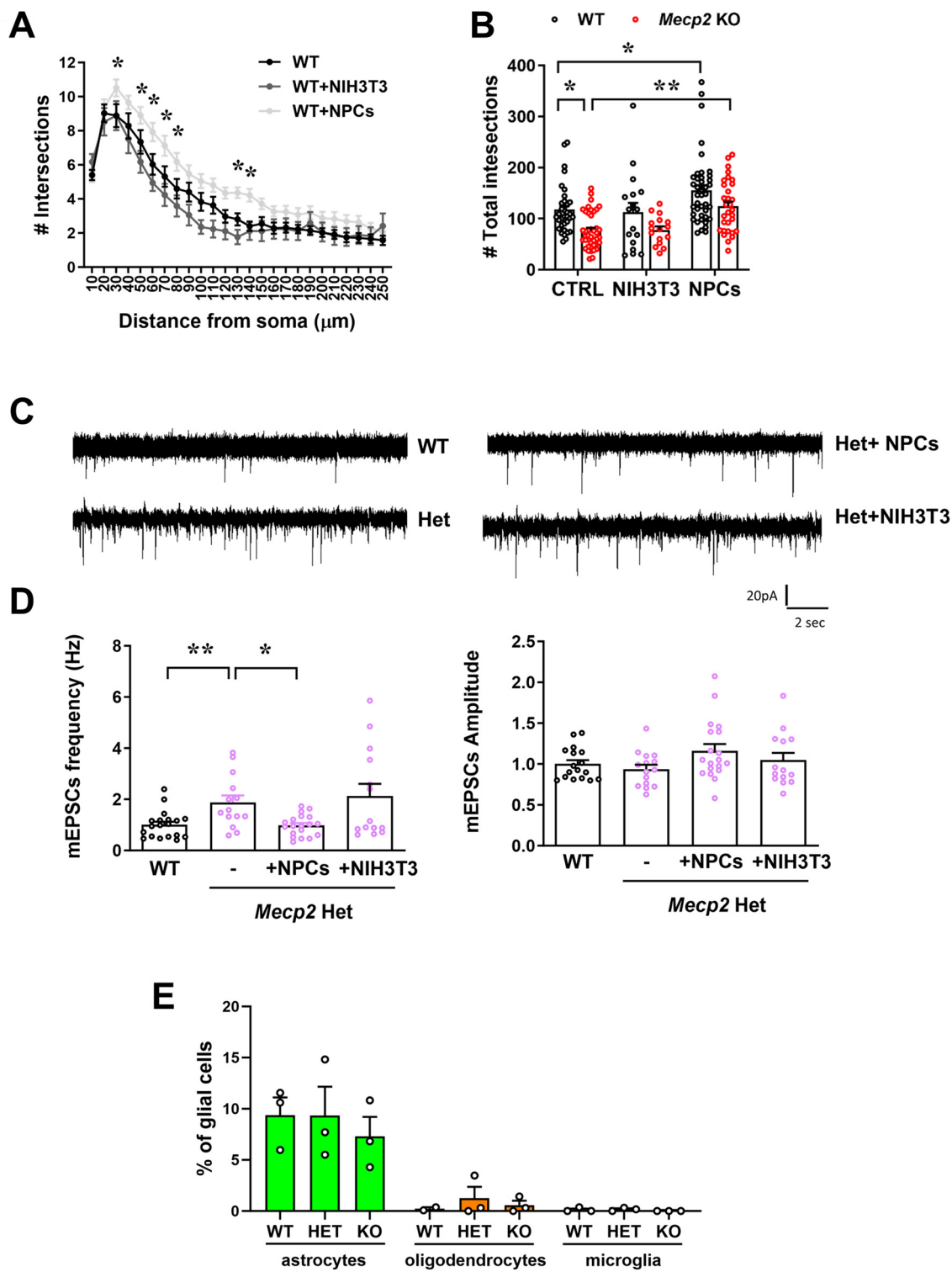


Expanded View Figures

Figure EV1. NPC effects on the morphological and functional properties of neurons.

(A) Sholl analysis reports the capacity of NPCs to increase dendritic complexity also in WT neurons. The graph depicts the mean $\pm$ SEM of the number of intersections of WT neurons cultured alone, or with NIH3T3 or NPCs from DIV0 to DIV7. $*p = 0.0194$ at 30 μm , $*p = 0.0270$ at 50 μm , $*p = 0.0045$ at 60 μm , $*p = 0.0074$ at 70 μm , $*p = 0.0256$ at 80 μm , $*p = 0.0262$ at 130 μm , $*p = 0.0070$ at 140 μm by two-way ANOVA followed by Tukey post-hoc test. $n = 33$ WT, $n = 17$ WT + NIH3T3; $n = 39$ WT + NPC. (B) The histogram shows the mean $\pm$ SEM of the total number of intersections calculated by Sholl analysis for WT and KO neurons cultured alone, or in culture with NIH3T3 or NPCs from DIV0 to DIV7. $*p = 0.0135$ WT vs KO, $*p = 0.0275$ WT vs WT+NPCs, $**p = 0.0032$ KO vs KO+NPCs by two-way ANOVA followed by Tukey post-hoc test. $n = 33$ WT, $n = 17$ WT + NIH3T3; $n = 39$ WT + NPC; $n = 39$ KO; $n = 26$ KO + NIH3T3; $n = 32$ KO+NPCs. Neurons derived from at least 3 different mice/genotype. (C) Representative traces of excitatory postsynaptic current in miniature (mEPSCs) recorded in primary WT and Het neurons (left) and in Het neurons treated with NPC or NIH3T3 (right). (D) Histograms represent the mean $\pm$ SEM of the mEPSCs frequency (Hz) and amplitude (pA) both expressed as values normalized on the frequency of WT neurons or amplitude of WT neurons. $n = 26$ WT; $n = 24$ Het; $n = 19$ Het+NPCs; $n = 14$ Het+NIH3T3. Data derived from 3 independent experiments. $**p = 0.0025$ WT vs Het by Mann-Whitney test; $*p = 0.0405$ by one-way ANOVA followed by Dunn's test. (E) The graph reports the percentage of contamination $\pm$ SEM of astrocytes, oligodendrocytes and microglia in cortical neurons at DIV14, independently from the genotype. $n = 3$ for each genotype.



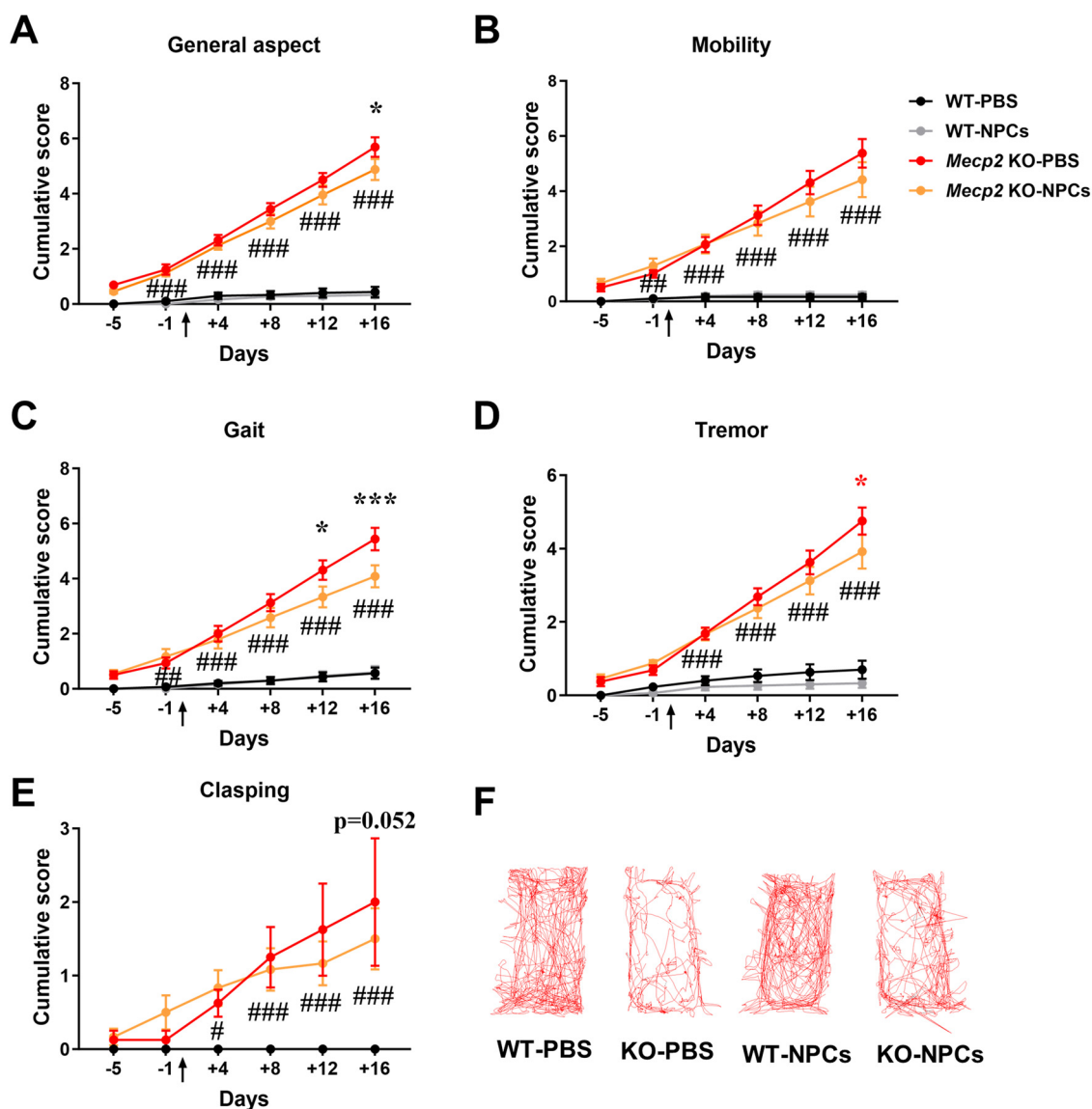


Figure EV2. Phenotypic characterization of NPC-transplanted *Mecp2* KO mice.

(A–E) Behavioral scoring was performed by a researcher blind to the treatment, assigning a score between 0 and 2 to general aspect (A), mobility (B), gait (C), tremor (D), and clasping (E). For each parameter, the graph reports its progression by a cumulative plot, in which the mean $\pm$ SEM of each value is obtained by summing the score of each day with those assigned the preceding days. Asterisks indicate a significant difference between KO + PBS and KO + NPCs; hashtags denote a difference between KO + NPC and WT mice. # $p = 0.0140$ for clasping, ## $p = 0.0010$ for gait, ### $p = 0.0022$ for mobility, ### $p < 0.0001$ for parameters, * $p = 0.0144$ for general aspect, * $p = 0.0224$ and *** $p = 0.0005$ for gait, * $p = 0.0367$ for tremor by two-way ANOVA followed by Tukey post-hoc test. $n = 15$ WT + PBS, $n = 15$ WT + NPCs, $n = 9$ KO + PBS, $n = 12$ KO + NPCs. (F) Representative traces of the distance travelled during the first day of NOR test by WT + PBS, KO + PBS, WT + NPCs and KO + NPCs mice. The corresponding data are reported in Fig. 4H.

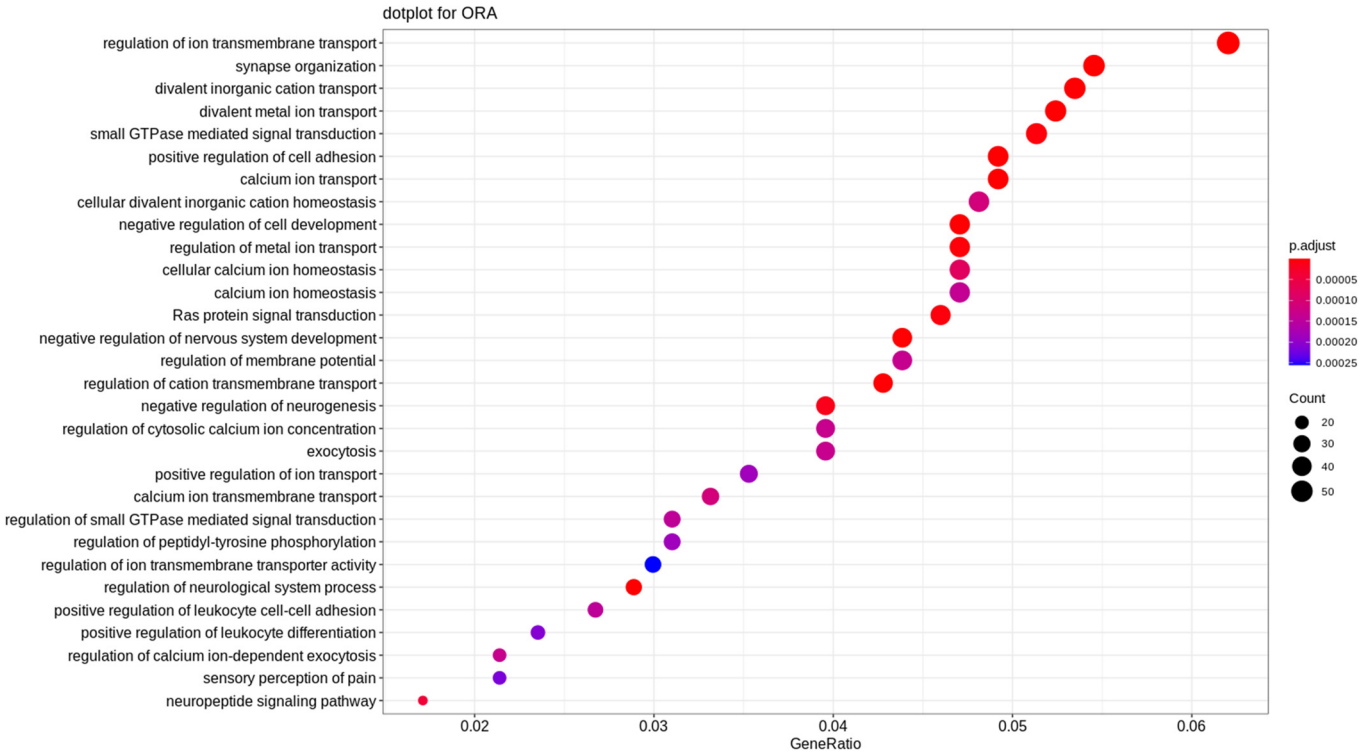


Figure EV3. Gene Ontology (GO) analysis.

(A) Dot plot of Gene Ontology (GO) enriched pathway analysis in the cerebellum, indicating the top 30 most enriched pathways of the comparison between KO versus WT samples. For a description of the statistical method implemented in ORA see Yu et al, 2012.

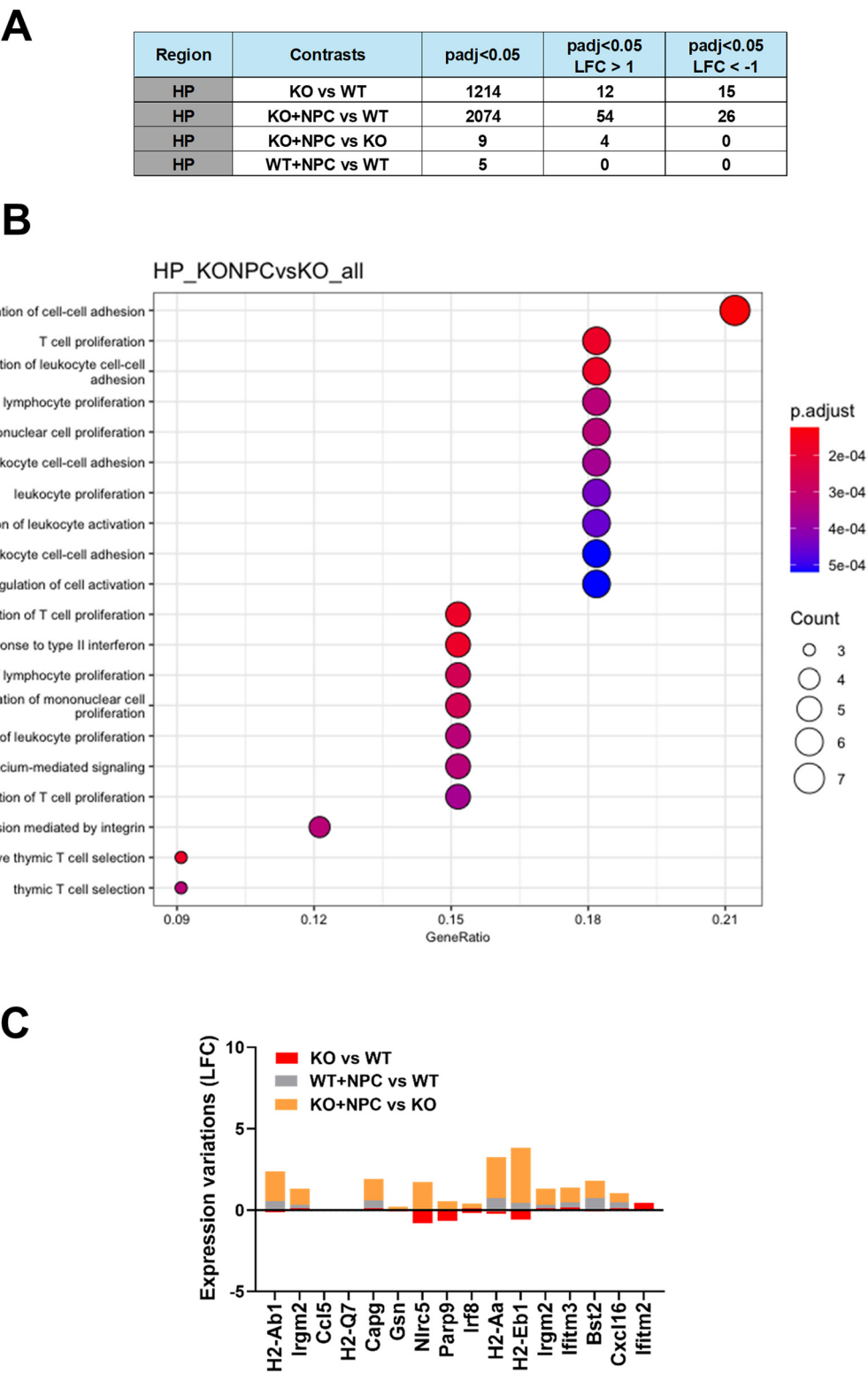


Figure EV4. Bulk RNA-sequencing indicated a mild upregulation of the IFN γ response in the hippocampus of KO mice after NPC transplantation. (A) The table reports the number of deregulated genes (DEGs) for the different comparisons, considering a $p_{\text{adj}} < 0.05$ and a $p_{\text{adj}} < 0.1$. The number of DEGs with a LogFoldChange lower than -1 (down-regulated genes) or LogFoldChange greater than 1 (upregulated genes) is also indicated. WT ($n = 6$), KO ($n = 5$), WT+NPCs ($n = 5$) and KO+NPCs ($n = 6$) hippocampi. (B) Dot plot of Gene Ontology (GO) indicating the top 20 most enriched pathways of the comparison between KO+NPCs versus KO samples. For a description of the statistical method implemented in DESeq2 or ORA see Boyle et al (2004) and Yu et al (2012), respectively. (C) The graph shows the LogFoldChange (LFC) of genes belonging to the GO pathway “Interferon- γ response” in the comparisons KO versus WT, WT + NPC versus WT, and KO+NPCs versus KO.

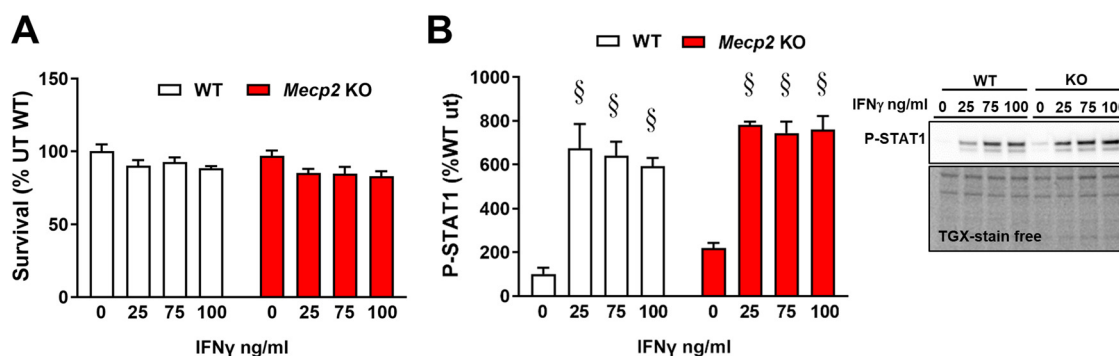


Figure EV5. IFN γ does not affect neuronal survival and activates its downstream kinase in vitro.

(A) The histogram represents the cell survival (% untreated WT neurons) $\pm$ SEM, assessed by MTT assay. IFN γ was added for 24 h in DIV13 primary neurons and three doses were tested: 25, 75, and 100 ng/ml. (B) The histogram reports the mean $\pm$ SEM of the levels of phosphorylated STAT1 after IFN γ treatment. Data are normalized to total protein content, visualized by a TGX stain-free technology. Representative bands of phosphorylated STAT1, and the corresponding lanes of TGX-stain-free gel, in WT and KO neurons treated or not with IFN γ are depicted. $\$p = 0.0003$ WT-UT vs WT 100 ng/ml and $\$p < 0.0001$ for the other comparisons, by two-way ANOVA followed by Tukey's post-hoc test. $\$$ denotes a significant difference respect to the corresponding untreated control of the same genotype. WT and KO neurons derived from 3 different mice/genotype.