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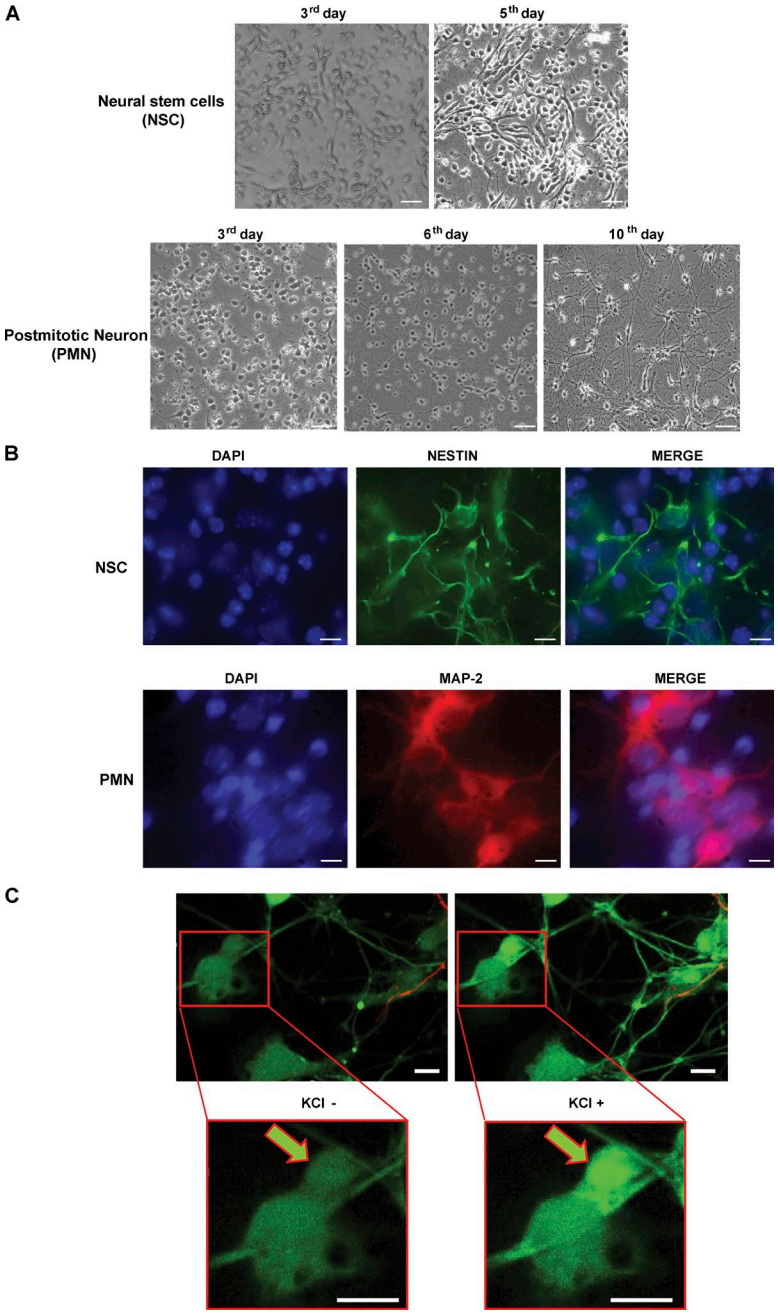

Figure S1. **Characterization of neural stem cells and PMNs. (A)** Phase contrast images of neural stem cells (NSCs) and PMNs. NSCs are shown on days 3 and 5 and PMNs on days 3, 6, and 10. Bars, 20 µm. **(B)** Staining of NSCs and PMNs with their respective markers: nestin (green) for NSCs and microtubule-associated protein 2 (MAP2; red) for PMNs. Bars, 10 µm. **(C)** Live-cell calcium imaging. Fluo-4 was used to measure intracellular calcium signal. KCl induces calcium influx (depolarization). Left: Before KCl. Right: After 12.5 mM KCl. Arrows indicate an excited cell. Bars, 5 µm.

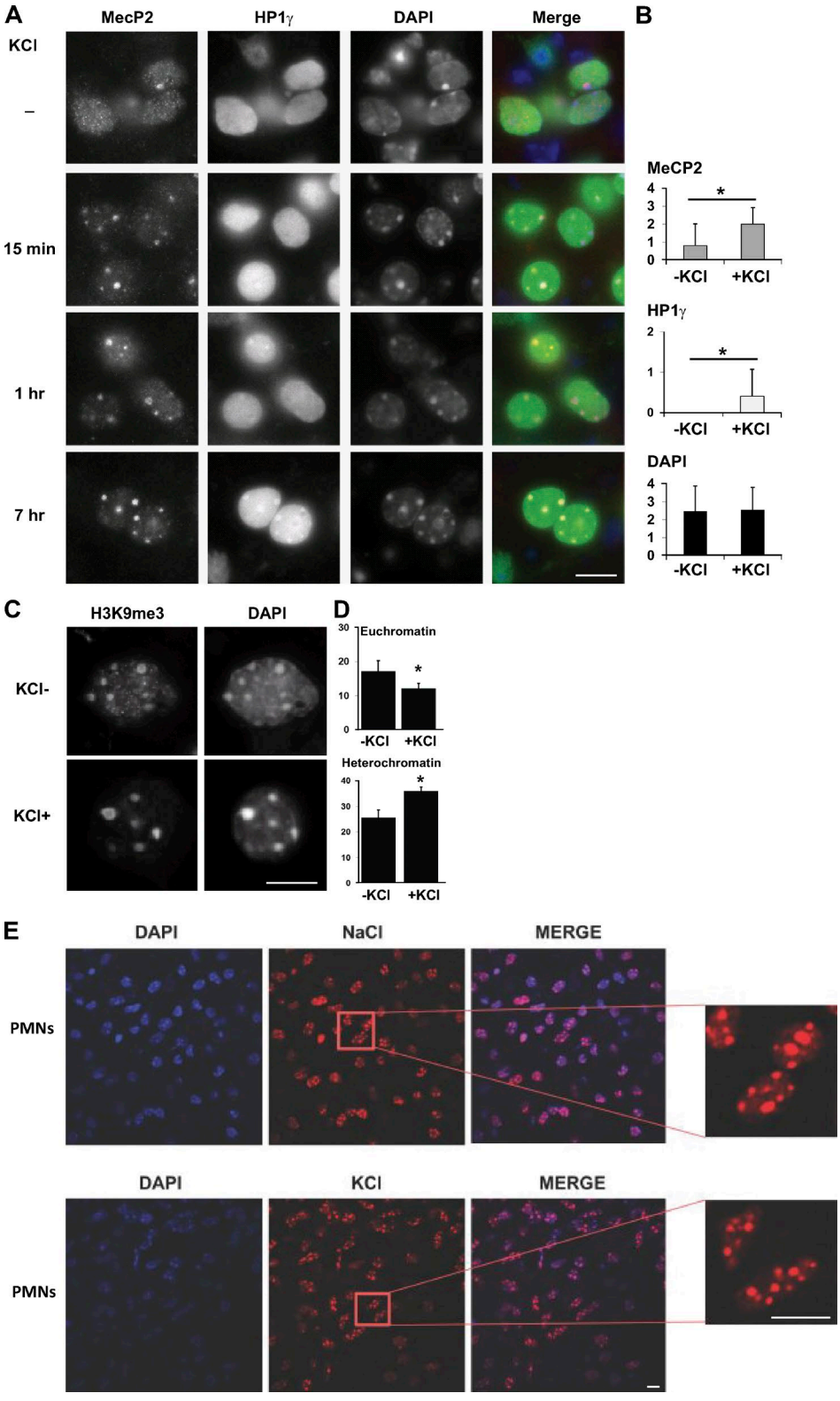

Figure S2. **Chromatin reorganization after neuronal stimulation in primary neurons. (A)** Chromatin rearrangements in primary PMNs before and after depolarization. Resting (top lane) and stimulated (15 min KCl, lane 2; 1 h KCl, lane 3; and 7 h KCl, lane 4) PMNs were coimmunolabeled with MeCP2 (left) together with HP1γ and counterstained with DAPI. Merged image is shown in color (right). This experiment was independently repeated at least twice. Bar, 5 µm. **(B)** Quantification of the immunofluorescence experiments. Error bars represent standard deviation. *, $P < 0.05$, two-tailed *U* test. **(C)** H3K9me3 in PMNs before and after KCl stimulation. Resting (top) and stimulated (bottom) PMNs were labeled with H3K9me3 (left). DAPI is shown on the right. Note diffuse staining in resting neurons compared with stimulated neurons, where H3K9me3 is concentrated in heterochromatin foci. This experiment was independently repeated at least twice. Bar, 5 µm. **(D)** Quantification of the immunofluorescence stainings image from C. Error bars represent standard deviations. *, $P < 0.05$, two-tailed *U* test. **(E)** HP1α immunostaining. No changes in HP1α chromatin organization after neuronal excitation by KCl was observed. Resting (NaCl, top) and stimulated (KCl, bottom) PMNs were labeled with HP1α (middle, red). DAPI (left, blue) and merge (right) are shown. Bars, 2 µm.

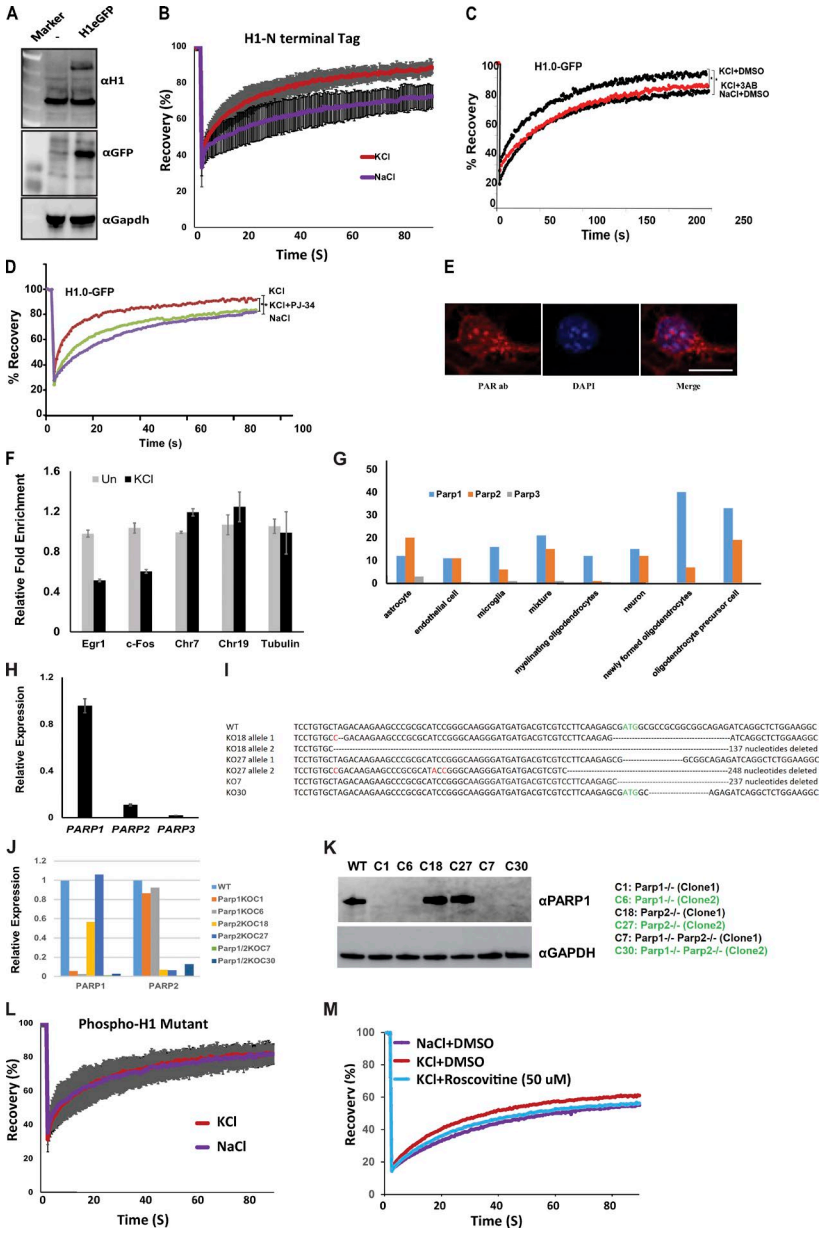

Figure S3. **PARP1 inhibitors retain H1 on chromatin after neuronal excitation. (A)** PMNs were transfected with or without H1-GFP expression plasmid. Approximately one million GFP-positive cells were FACS sorted and blotted for H1 (top), GFP (middle), and GAPDH (bottom). A similar number of control cells were used. **(B)** FRAP experiments (on at least 20–30 cells) for N-terminally tagged GFP-H1e in resting (NaCl) or depolarized (KCl) WT PMNs. Error bars represent standard deviation ($P < 0.001$). **(C)** FRAP analysis of (at least 20–30 cells) H1.0-GFP in depolarized (KCl) or unstimulated (NaCl) PMNs with and without the PARP inhibitor 3AB (*, $P < 0.05$, two-tailed Student's *t* test). **(D)** FRAP analysis of H1.0-GFP (on at least 20–30 cells) in depolarized (KCl) or unstimulated (NaCl) PMNs with and without the PARP inhibitor PJ34 (*, $P < 0.05$, two-tailed Student's *t* test). **(E)** PMNs derived from WT cells were treated with 12.5 mM KCl for 1 h and stained with PAR antibody. The high-resolution image of KCl stimulated PMNs nucleus showing the localization of PAR modification. Cells were counterstained with DAPI to visualize DNA. Bar, 5 µm. **(F)** Selective clearance of IEG promoters from histone H1 in stimulated PMNs. ChIP assays were performed to analyze histone H1 occupancy in *c-Fos*, *Egr1*, and *Tubulin* promoters as well as in silent loci in chromosome 7 (Chr7) and chromosome 19 (Chr19). Error bars represent standard deviation. The experiment was independently repeated at least twice. **(G)** Publicly available expression datasets for different PARPs (obtained from the European Bioinformatics Institute) reveal that expression of PARP1 and PARP2 dominate neuronal tissues. **(H–K)** Generation and validation of PARP knock-out cells. PARP expression in PMNs (H). Total RNA was isolated from WT cells, and quantitative PCR was performed to analyze the expression (mRNA) of PARP1, PARP2, and PARP3. Error bars represent standard deviations. The experiment was independently repeated at least three times. PARP2 genomic validation (I). Total genomic DNA was isolated from WT and the different KO clones as indicated on the left. Approximately 600 nt flanking the "PARP2 CRISPR KO guide site" were amplified using PCR and sequenced. The green font shows the translation start site in the *Parp2* gene. A similar analysis was done for *Parp1*. RNA validation (J). Quantitative PCR was performed to analyze expression level of *Parp1* and *Parp2* in WT cells and in the different KO cells. The experiment was independently repeated at least three times. Protein validation (K). Protein extracts from WT and the different KO clones, as indicated on the right, were subjected to Western blot analysis using PARP1 antibody. GAPDH was used as a loading control. The cell lines labeled in green were used for subsequent experiments. **(L)** FRAP experiments (on at least 20–30 cells) for GFP-H1e in which all phosphorylation sites have been converted to alanines in resting (NaCl) or depolarized (KCl) WT PMNs. Error bars represent standard deviation. The typical increased mobility of H1 after KCl stimulation is prevented in the phospho-mutated H1. **(M)** FRAP experiments (on at least 20–30 cells) for GFP-H1e in resting (purple), KCl-stimulated (red), or in KCl-stimulated PMNs pretreated with 50 µM roscovitine (blue). The typical increased mobility of H1 after KCl stimulation is almost completely prevented by 50 µM roscovitine.

Table S1. **Primer sequences used in real-time PCR and ChIP**

Experiment	Name	Sequence (5′–3′)
Real-time PCR	*Cfos*	F, ATCGGCAGAAGGGGCAAGTAG; R, GCAACGCAGACTTCTCATCTTCAAG
	Cjun	F, CATGAGGAACCGCATCGCTGCCTCCAAGT; R, GCGACCAAGTCCTTCCCACTCGTG CACACT
	Egr1	F, AACACTTTGTGGCCTGAACC; R, GATGAAGCAGCTGGAGAAGG
	Gapdh	F, ATGTTCCAGTATGACTCCACTCACG; R, GAAGACACCAGTAGACTCCACGACA
ChIP	*Cfos* promoter	F, TGGACTTGACTGGGGGTCTG; R, CAGGTCCACATCTGGCACAG
	C*jun* promoter	F, CCAGCCCTGAACTTGAGC; R, ATCCAGCCTGAGCTCAACAC
	Egr1 promoter	F, CAGAAGCCCTTCCAGTGTCG; R, GATGGGTAGGAGGTAGCCAC
	β-Tubulin promoter	F, TAGAACCTTCCTGCGGTCGT; R, TTTTCTTCTGGGCTGGTCTC
	Chr19 NegChIP	F, GCGACTCTGAAAATTCCCGTC; R, AGCGCGTTCTTCATAACCCA
	Chr7 NegChIP	F, TGTGCGAAGGGCGGATCTA; R, CACTATATGGCCCTGCCTATTCTC

F, forward; R, reverse.