Supplementary Figures and Tables for:

PARP6 is a Regulator of Hippocampal Dendritic Morphogenesis

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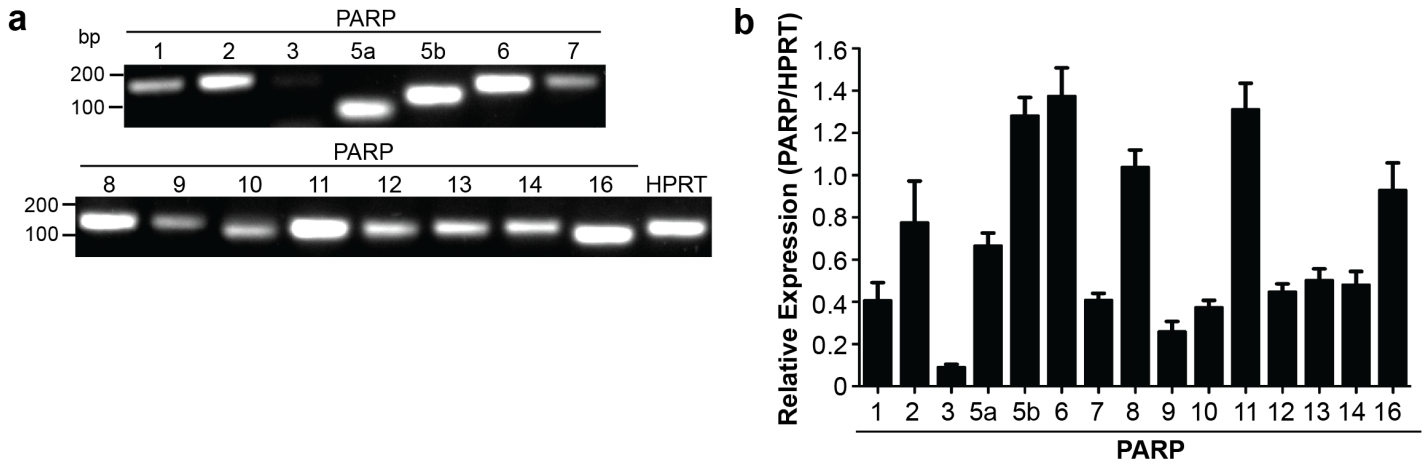
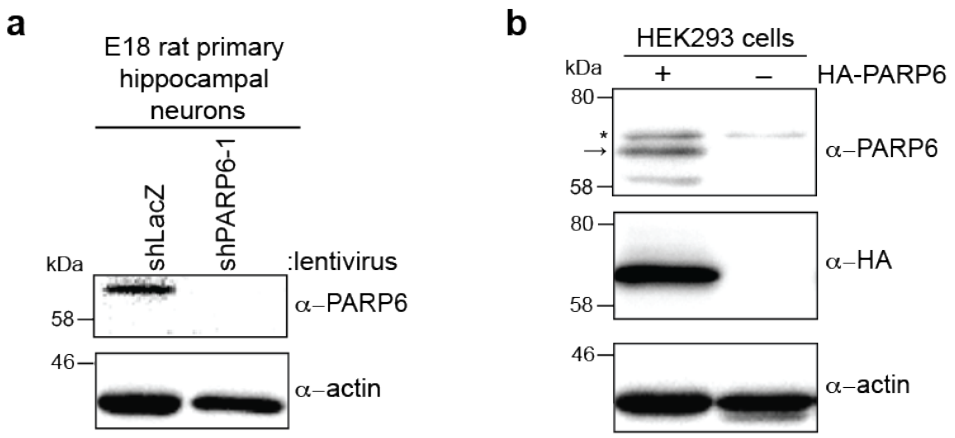


Supplementary Figure 1. PARP6 is the most abundant PARP in E18 rat hippocampal neurons.

(**a**) PARP family-wide mRNA expression profiling. RT-PCR was performed on cDNA from E18 rat hippocampal neurons. PARP-specific primer sequences used for RT-PCR are shown in **Supplementary table 1**. Hypoxanthine phosophoribosyltransferase (HPRT) served as a control.

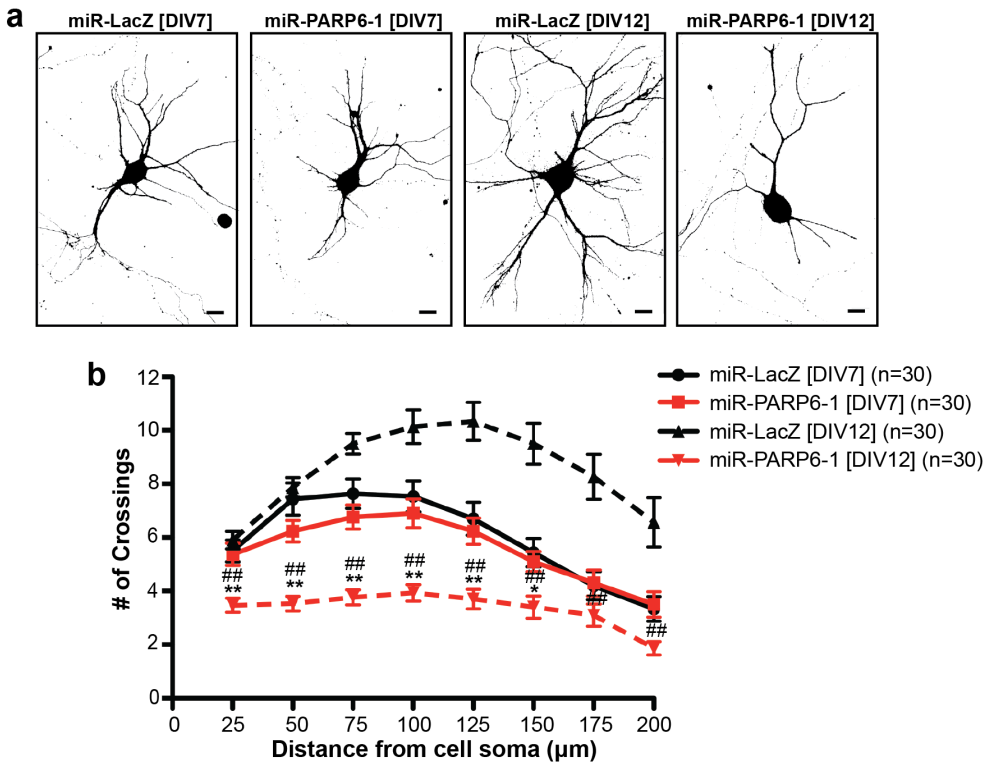
(**b**) Quantification of results shown in **a**. Data were obtained from three independent experiments. Error bars represent SEM.



Supplementary Figure 2. Specific detection of PARP6 using an anti-PARP6 antibody.

(**a**) Validation of a commercially available PARP6 antibody using lentiviral mediated shRNA-based knockdown. E18 rat hippocampal neurons were transduced on day *in vitro* 0 (DIV0) with lentiviruses expressing either an shRNA targeting PARP6 (shPARP6-1) or LacZ (shLacZ). Neurons were harvested on DIV7 and proteins were resolved by SDS/PAGE and detected by Western blot with anti-PARP6 (Sigma; HPA026991) and anti-actin antibodies.

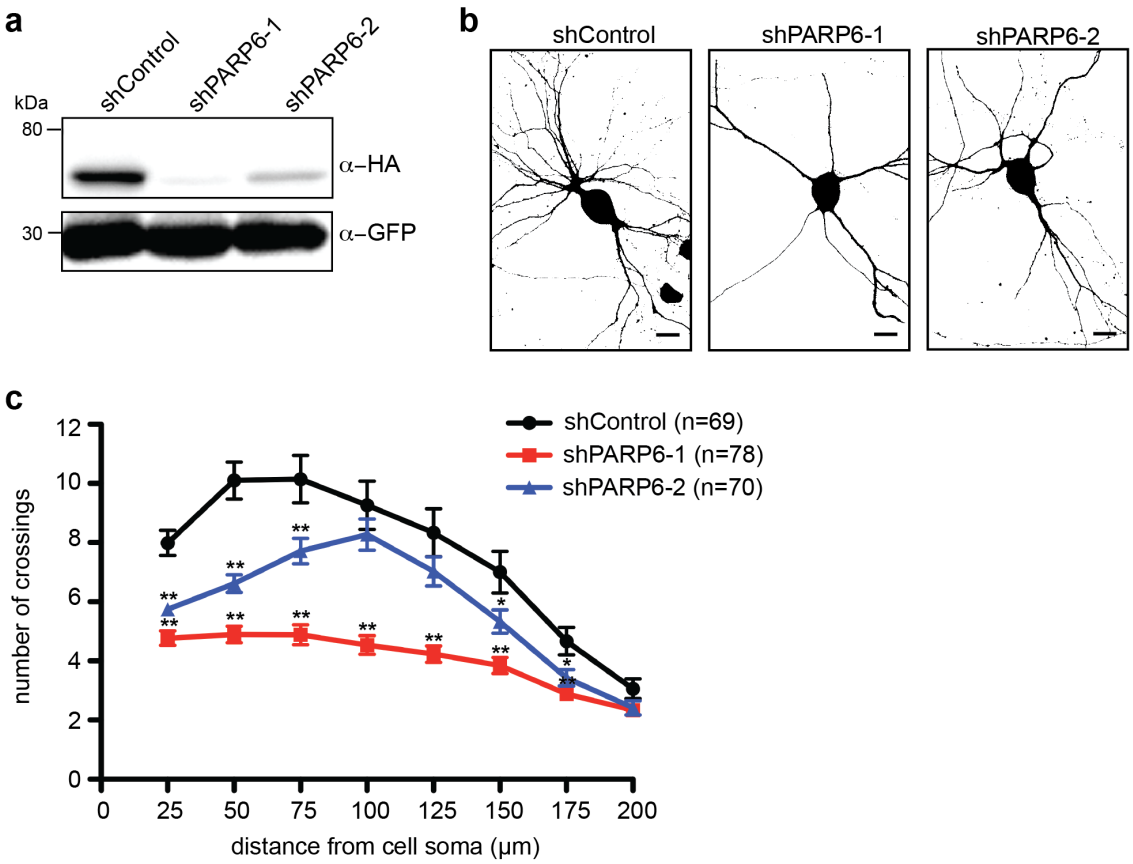
(**b**) Sigma anti-PARP6 detects HA-PARP6 overexpressed in HEK 293T cells. HEK 293T cells were transfected with HA-PARP6. Proteins were resolved by SDS/PAGE and detected by Western blot with anti-PARP6 (Sigma; HPA026991), anti-HA, and anti-actin antibodies. →denotes expected size of HA-PARP6 protein (~69 kDa), *denotes putative higher-molecular weight full-length splice variant of PARP6 protein (~71 kDa).



Supplementary Figure 3. PARP6 knockdown reduces dendritic growth and maintenance.

(**a**) E18 primary rat hippocampal neurons were transfected with miRNA-based knockdown plasmids on DIV6 and fixed on either DIV7 or DIV12. Shown are representative binary images generated from fluorescent images. Scale bar, 20 μm.

(**b**) Quantification of results in **a** using Sholl analysis. Error bars represent SEM. *p<0.05, **p<0.001 (one-way ANOVA followed by Tukey's HSD test) compared to miR-LacZ [DIV7]; ##p<0.001 (one-way ANOVA followed by Tukey's HSD test) compared to miR-LacZ [DIV12].

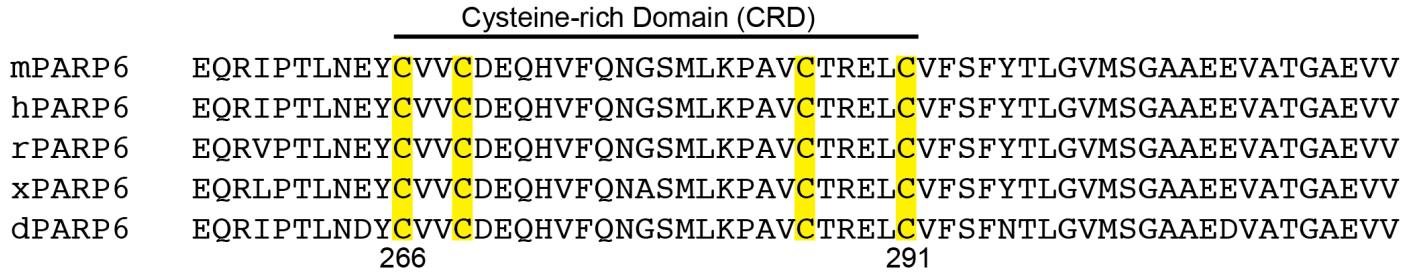


Supplementary Figure 4. PARP6 regulates dendritic complexity in primary hippocampal neurons.

(**a**) shRNA-based plasmids effectively knockdown PARP6 protein levels. HEK 293T cells were co-transfected with HA-PARP6 and GFP-expressing shRNA-based knockdown plasmids targeting rat PARP6 (shPARP6-1 or 2) or a non-targeting control (shControl). Proteins were resolved by SDS/PAGE and detected by Western blot with anti-HA and anti-GFP antibodies.

(**b**) Knockdown of PARP6 using shRNA-based plasmids decreased dendritic complexity. E18 primary rat hippocampal neurons were transfected with shRNA-based knockdown plasmids on DIV7 and fixed on DIV12. Shown are representative binary images generated from fluorescent images. Scale bar, 20 μm.

(**c**) Quantification of results in **b** using Sholl analysis. Error bars represent SEM. *p<0.05, **p<0.001 (one-way ANOVA followed by Tukey's HSD test) compared to shControl.



Supplementary Figure 5. Alignment of PARP6 orthologues demonstrates conservation of the cysteine-rich domain (CRD). mPARP6 = Mus musculus PARP6 (NM_001205239); hPARP6 = Homo sapiens PARP6 (NM_020214); rPARP6 = Rattus norvegicus PARP6 (NM_001106828); xPARP6 = Xenopus tropicalis PARP6 (NP_001090857); dPARP6 = Danio rerio PARP6 (XP_009296376).

Supplementary Table 1: PARP-specific primer sequences for endpoint-PCR expression profiling

PARP	Forward Primer Sequence	Reverse Primer Sequence	Amplicon Length
1	TCTACTTTGCTGATATGGTGTCC (bp 2746-2768)	TGGGTAACTTGCTGATGTGAG (bp 2889-2869)	144
2	GAAATTGCCCTTAAGCTGGTG (bp 980-1000)	TGGGTAGACTGTAAGTACTGGG (bp 1128-1107)	149
3	CTCGGAGAACAGCAAGTCAG (bp 1349-1368)	GGGTGGACTCTTCAAGCTG (bp 1490-1472)	142
5a	CACCGCCACAAGTTAATCAAG (bp 3218-3238)	AAGGTCAGATACGGATTGGTTC (bp 3291-3270)	74
5b	AGCGAGAACAGATCACCTTG (bp 2684-2703)	ACCCCTTTAATGAGCCTGTG (bp 2789-2770)	106
6	CTCCGTGTCCATCAGAGAATATG (bp 645-667)	ACCGTAGCCTCAACACAATAG (bp 781-761)	137
7	GTATGCCTGTCCTGATTCCTG (bp 586-606)	TCTGCTACTGTGCCATTGATG (bp 727-707)	142
8	ATGTTAGCCTTCAACCCCAG (bp 2158-2177)	CAGAAGTGGATGAGCAAGGG (bp 2307-2288)	147
9	TGGGTGTAGAAGTGGGAGAG (bp 1761-1780)	TTGAACTTGTCTGGAGCTGAG (bp 1910-1890)	150

10	GAAATGGTGCTGTCAATGGAG (bp 1210-1230)	CCGAAAGCCAGTCACATCTAC (bp 1335-1315)	126
11	TGACTGGAGAATCAATGGTGTG (bp 956-977)	AAGCTGACCCCATGAATCTG (bp 1093-1074)	138
12	TGTTTGTTAAGACCAAGCACAGA (bp 3011-3033)	AGGCCACTATGCTCCTCTGA (bp 3152-3133)	142
13	CGGTGGCAGATGTTCGTATAT (bp 1840-1860)	GGTAGGAGTCACAGGTCATTTTC (bp 1984-1962)	145
14	GACATTGGTATCTGCCGTAGG (bp 5387-5407)	TCAAGTGGTTGGCTCTCAAG (bp 5535-5516)	149
16	CCGCCTAGAGAACTTCCATTC (bp 582-602)	CCGTGAGGACTATAAATGAGGG (bp 710-689)	129

Supplementary Table 2: PARP6 RNAi sequences

RNAi constructs	**Target Sequence**	**Species Specificity**
miR-LacZ	GACTACACAAATCAGCGATTC	N/A
miR-PARP6-1	TTGCACCATGAAGAACCCAAA (bp 1125-1145)	Mouse, Rat
miR-PARP6-2	GGGCTCATATTTGGAAATCAA (bp 1674-1694)	Mouse, Rat

miR-PARP6-3	CTACACTAAACTGCAGCTGCA (bp 1959-1979)	Rat
shControl	ACTACCGTTGTTATAGGTGTC	N/A
shPARP6-1	TTGCACCATGAAGAACCCAAA (bp 1125-1145)	Mouse, Rat
shPARP6-2	GATGACCCAGGGCTCATATTT (bp 1665-1685)	Mouse, Rat