

Supplementary information

Transcriptional repression of *PTEN* in neural cells using CRISPR/dCas9 epigenetic editing

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Supplementary Table 1. gRNA recognition sequences for *PTEN* repression. The numbering of the start and end of each gRNA is given according to the Genome Reference Consortium Human Build 38 (GRCh38). PAM: protospacer-adjacent motif; F: forward strand guide; R: reverse strand guide. Location refers to the genomic position of the gRNA binding site in base pairs upstream (-) or downstream (+) the transcription start site of *PTEN* transcript NM_000314.8 in GRCh38.

gRNA	crRNA recognition sequence	PAM	F/R	Start	End	Location
A	GAGGATAACGAGCTAAGCCT	CGG	R	87863256	87863237	-368
B	GCGCAGAGTCCCAAGCCGC	AGG	R	87863383	87863364	-241
C	GCTGCGGCAGGATACGCGCT	CGG	F	87863580	87863599	-45
D	GCGCCTGTGAGCAGCCCGG	GGG	F	87863748	87863767	+123

Supplementary Table 2. Analysis of predicted off-target binding sites of *PTEN* gRNAs. In the off-target recognition sequence, mismatches to the cognate gRNA sequence are shown in lower case and underlined. Position refers to chromosomal location in GRCh38. Distances from the TSS of off-target genes are approximate and refer to annotated RefSeq transcripts. Literature-curated TF binding sites and associated genes are from the PAZAR and JASPAR databases. Regions of H3K4me3, H3K4me1 and H3K27ac enrichment are obtained from the ENCODE project. Chr: chromosome; TF: transcription factor; TSS: transcription start site.

gRNA	Off-target recognition sequence	Chr	Position	Direction	Mismatches	Proximity to regulatory elements	Potential gene affected
A	GAGGATAACGAGC <u>cc</u> AGCC <u>a</u> GGG	19	16105684	+	3	None	None
B	G <u>a</u> GCA <u>t</u> A <u>a</u> TCCCCAAGCCGCTGG	3	119690285	-	3	12 kb upstream of <i>COX17</i> TSS; overlaps CTCF TF binding sites for <i>COX17</i> ; in DNase I hypersensitive region	<i>COX17</i>
	GC <u>t</u> CAGAGTCCCCAAG <u>g</u> C <u>c</u> CTGG	3	140566600	+	3	None	None
	GCGC <u>t</u> GAGTCCCCAAGC <u>a</u> G <u>a</u> TGG	4	8738833	+	3	None	None
	G <u>t</u> GCAGAGTCCCCAAG <u>g</u> CG <u>g</u> CGG	5	73447021	+	3	1.5 kb downstream of <i>FOXD1</i> TSS; in CpG island; overlaps E2F4 and EGR1 TF binding sites for <i>FOXD1</i> ; in DNase I hypersensitive region; in region of H3K27ac, H3K4me3, H3K4me1 enrichment	<i>FOXD1</i>
	GCGC <u>a</u> AGTCCC <u>a</u> AGCCGCCGG	1	923628	+	3	2 kb upstream of <i>SAMD11</i> TSS; in CpG island; overlaps EGR1 TF binding sites for <i>SAMD11</i> ; in region of H3K4me3, H3K4me1 enrichment	<i>SAMD11</i>
	GC <u>t</u> CAGAG <u>a</u> CCCCAAGCC <u>t</u> CGGG	1	3983348	-	3	None	None
G <u>t</u> GC <u>t</u> GAGTCCCCAAGCC <u>c</u> CAGG	9	136764513	+	3	None	None	
C	GCTGCG <u>t</u> CgGGATAgGCGCTGGG	16	89721972	+	3	1 kb upstream of <i>VPS9D1</i> TSS; overlaps SMARCA4 TF binding	<i>VPS9D1</i> ; <i>ZNF276</i>

						site for VPS9D1; 1 kb downstream of <i>ZNF276</i> TSS; overlaps STAT1 TF binding site for <i>ZNF276</i> ; in region of H3K27ac, H3K4me3, H3K4me1 enrichment	
	GCTGaGGCAGGAgACTcCGCTTGG	1	1497763	+	3	None	None
	GCTGCaGCAGGATACaCcCTGGG	1	18823163	+	3	None	None
	GCTGgGGCAGGATAaAtCGCTTGG	13	34275296	+	3	None	None
	GCTGCGGCAGGAgAatCGCTTGG	13	110571118	+	3	None	None
	GCTGaGGCAGGAgACTcCGCTTGG	10	86424569	+	3	None	None
	GCTGaGGCAGGAaACTcCGCTTGG	10	110432686	-	3	None	None
	GCTGaGGCAGGATAaAtCGCTCGG	18	50856030	-	3	None	None
	GCTGCaGCAtGATACGtGCTAGG	11	50193345	+	3	None	None
D	aCGCgTGTcAGCAGCCGCGGCGG	7	150975260	+	3	3 kb downstream of <i>KCNH2</i> TSS; overlaps EGR1 and TFAP2C TF binding sites for <i>KCNH2</i> ; in region of H3K27ac, H3K4me3, H3K4me1 enrichment	<i>KCNH2</i>
	GCCcCTGTGAGCAGCctCtGTGG	4	8025678	-	3	None	None
	GCCcCTGTGAGCAGctGCaGGGG	16	897591	+	3	None	None
	GCCcCTGTGgGCAGCCaCGGGG	1	2177765	-	3	None	None
	GCGCCgGcGAGCAGCCcCGGGG	19	1851994	+	3	0.4 kb upstream of <i>KLF16</i> TSS; in CpG island; overlaps SMARCA4 TF binding site for <i>REXO1</i> ; in DNase I hypersensitive region	<i>KLF16</i> ; <i>REXO1</i>

GCGCCaGTGAGCAGgCGCaGCGG	17	17164604	+	3	60 kb downstream of <i>MPRIP</i> TSS; in CpG island	<i>MPRIP</i>
GCTcCTGTcAGCAGctGCGGAGG	10	129709862	-	3	None	None
GCGCCTGTGAGCAGctGgaGAGG	14	54348869	+	3	45 kb upstream of <i>CDKN3</i> TSS; overlaps CTCF TF binding site for <i>CDKN3</i> ; in DNase I hypersensitive region; in region of H3K4me1 enrichment	<i>CDKN3</i>

Supplementary Table 3. shRNA recognition sequences for *PTEN* repression. Numbering refers to start position relative to the TSS of *PTEN* mRNA transcript variant 1 (Genbank accession number NM_000314.8) in mRNA not DNA. nt: nucleotide.

shRNA	Recognition sequence	Location in <i>PTEN</i> transcript
+874	GCAGAAACAAAAGGAGATATCA	Exon 1
+1436	GATGATGTTTGAAACTATTCCA	Exon 5
+1608	GTAGAGTTCTTCCACAAACAGA	Exon 6
+2022	GATGAAGATCAGCATTCACAAA	Exon 9 (1 nt mismatch)

Supplementary Table 4. TaqMan Gene Expression Assays used for qRT-PCR in *PTEN* repression experiments. For each gene, the TaqMan Assay ID, targeted RefSeq transcripts and reporter dye are listed.

Gene	Assay ID	RefSeq sequence(s)	Dye
<i>PTEN</i>	Hs02621230_s1	NM_000314.8 NM_001304717.2 NM_001304718.1	FAM-MGB
<i>GAPDH</i>	Hs02786624_g1	NM_001256799.2 NM_001289745.1 NM_001289746.1 NM_002046.5	FAM-MGB
<i>SAMD11</i>	Hs00942141_m1	NM_152486.2	FAM-MGB
<i>KLF16</i>	Hs00259103_m1	NM_031918.3	FAM-MGB

Supplementary Table 5. Primers used for SYBR Green qRT-PCR in *PTEN* repression experiments.

Gene	Forward primer	Reverse primer
<i>COX17</i>	GTCATAGCTGCTTTTGGCG	TCACACAGCAGACCACCATT
<i>FOXD1</i>	ATTGAACCCGAGAACGTCCG	TCAGATGCGTGCGTTACAGA
<i>VPS9D1</i>	CGCCATGAAGCTTGCCAAC	ATGCTCCTCAGGTATTCCGT
<i>ZNF276</i>	GGGTCCTCTAGGAACCTCGG	GACGACCCAGGAGACAGGAA
<i>KCNH2</i>	CAGCTCGAGCCAGGCAG	ATGAAGCCAGGGTGGTTGTG
<i>REXO1</i>	TCCAGGACAATGTGGATGGG	GTCTTGGCGTCTTCTCGAAC
<i>MPRIIP</i>	CACCTGCCTACGTGGACTC	CCAGGGTGGCATATTGCCTG
<i>CDKN3</i>	AGCCGCCAGTTCAATACAA	TCACTCGTGACAAAGATAGCCA
<i>GAPDH</i>	CTTAGCACCCCTGGCCAAG	GGCAGGGATGATGTTCTGGA
<i>Pten</i> (rat)	TTGAAGACCATAACCCACCACAG	CACAAATCATTACACCAGTCCGTC
<i>Ppia</i> (rat)	AGCATACAGGTCTTGGCATC	TTCACCTTCCCAAAGACCAC

Supplementary Table 6. Antibodies used for Western blotting in *PTEN* repression experiments. For each antibody, the isotype, manufacturer, catalogue number, molecular weight of the antigen and dilution factor in *PTEN* repression experiments are listed.

Antibody	Isotype	Manufacturer	Cat. no.	Molecular weight	Dilution
PTEN	Rabbit IgG	CST	9559	54 kDa	1/1000
GAPDH	Rabbit IgG	CST	2118	37 kDa	1/5000
Rabbit-HRP	Goat IgG	Jackson ImmunoResearch	111-035-144	—	1/1000
Mouse-HRP	Goat IgG	Jackson ImmunoResearch	115-035-003	—	1/1000

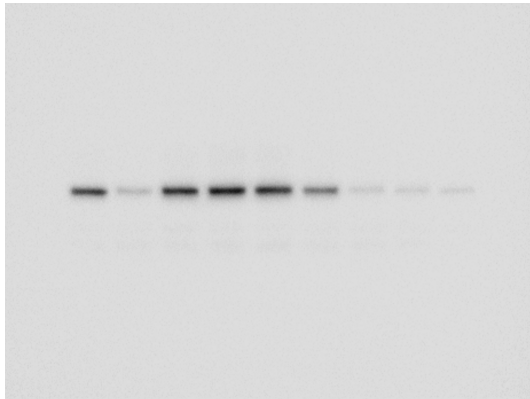
Supplementary Table 7. Primers used for ChIP-qPCR of *PTEN* and *GAPDH* regulatory regions.

Gene	Forward primer	Reverse primer	Product size
<i>PTEN</i>	ATGTGGCGGGACTCTTTATG	GCGGCTCAACTCTCAAACCTT	119 bp
<i>GAPDH</i>	TACTAGCGGTTTTACGGGCG	GAGGCTGCGGGCTCAATTT	137 bp

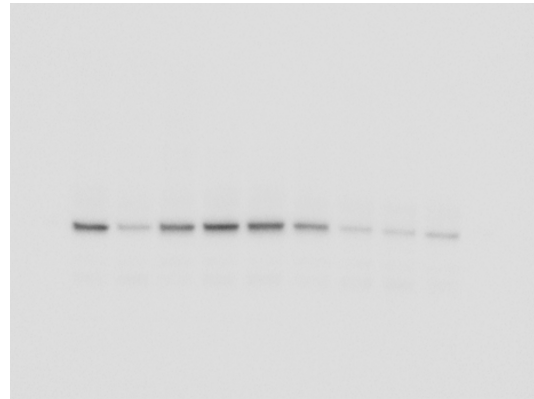
Supplementary Table 8. Antibodies used for immunofluorescence in *PTEN* repression experiments. For each antibody, the isotype, manufacturer, catalogue number and dilution factor in *PTEN* repression experiments are listed.

Antibody	Isotype	Manufacturer	Cat. no.	Dilution
GFP	Mouse IgG	Roche	11814460001	1/500
Nestin	Rabbit IgG	Biologend	841901	1/500
B-III tubulin	Rabbit IgG	Biologend	802001	1/500

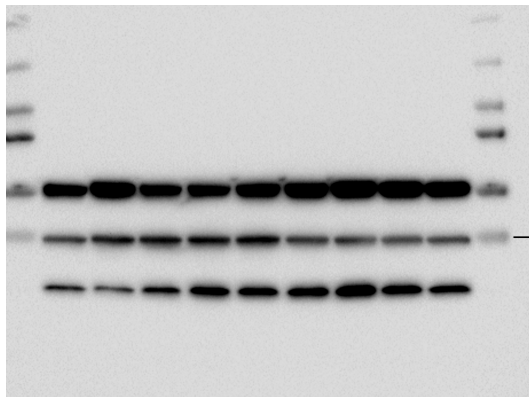
HEK 293T
PTEN



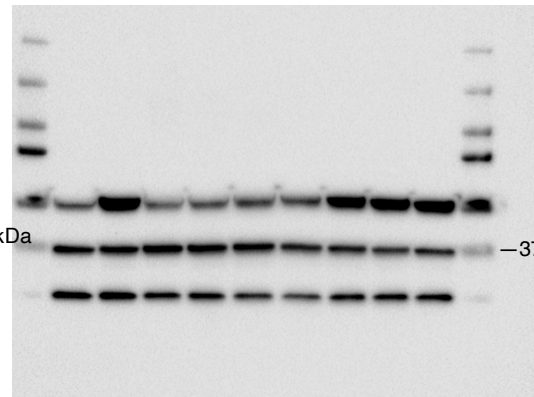
hMPCs
PTEN



HEK 293T
GAPDH



hMPCs
GAPDH



Supplementary Figure 1. Uncropped images of Western blots from Figure 2. GAPDH blots show Bio-Rad Precision Plus Protein Kaleidoscope Prestained Protein Standards with 37 kDa standard annotated.