Supplementary materials

P11 loss-of-function is associated with decreased cell proliferation and neurobehavioral disorders in mice

Running title: P11 and depression, anxiety and cognitive disorders

Guosheng Liu, Yabo Wang, Weixin Zheng, Hanhua Cheng*, Rongjia Zhou*

Hubei Key Laboratory of Cell Homeostasis, College of Life Sciences, Wuhan University, Wuhan 430072, P. R. China

*Corresponding authors: Professors Rongjia Zhou and Hanhua Cheng, College of Life Sciences, Wuhan University, Wuhan 430072, P. R. China, Fax: 0086-27-68756253, E-mail: rjzhou@whu.edu.cn, hhcheng@whu.edu.cn

The supplementary material files include:
One figure and two tables.

Figure S1

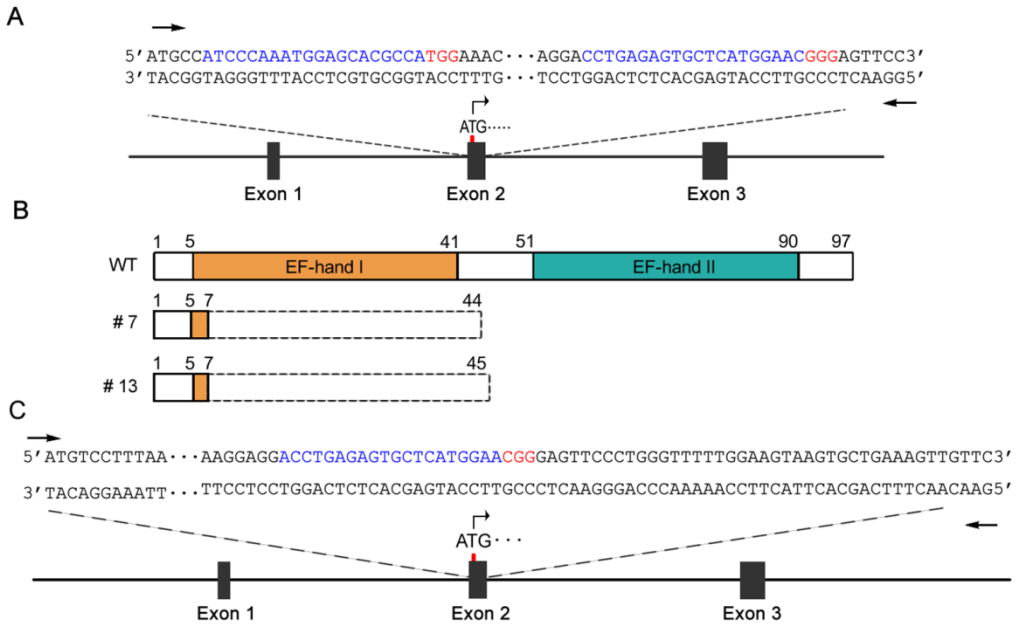

Fig. S1. Targeted disruption of *P11* in mice and in MEF cell lines

A. Schematic representation of gRNAs targeting the *P11* locus. The translation start codon ATG is indicated by an arrow, coding exons are numbered from 2 to 3, and exon 1 is a non-coding exon. Two gRNAs (blue) are designed to target open reading frame (ORF) in exon 2. PAMs, protospacer adjacent motif, are highlighted in red. Arrows indicate primer positions for genotyping of progeny.

B. Schematic diagram of protein coding regions of wild type P11 and predicted truncated mutants. The conserved domains (EF-hand I and EF-hand II) are indicated in boxes (solid lines). Frameshift sequences are shown in boxes with dotted lines. The numbers refer to the amino acid positions.

C. Schematic representation of gRNAs targeting the *P11* locus in MEF cell lines. As shown in Fig. S1A, gRNA is designed to target exon 2.

Table S1. The primers and conditions used in the study

PCR primers for identification of on-target indel mutations of *P11* in mice

Primer names	**Primer sequences (5'-3')**	**PCR conditions**
Crispr-mouse	F: ATGTCCTTTAACAGGTTTCGA	94°C, 30 s; 60°C, 30 s;
	R: GACTTTCAGCACTTACTTCCA	72°C, 30 s; 36 cycles

PCR primers for potential off-target CDS for *P11*-gRNA1 in mice

Gene names	**Primer sequences (5'-3')**	**PCR conditions**

Wdr7	F: ATGTATGCATGTGTGCATGC R: TCTCACTCCCTTCAAGAGCT	94°C, 30 s; 56°C, 30 s; 72°C, 30 s; 36 cycles
Robo4	F: GGTGCTGGAGAACTCAGTAC R: ACAGCACTACTGGCAATGAC	94°C, 30 s; 60°C, 30 s; 72°C, 30 s; 36 cycles
Tmc4	F: CTGTGGCCTGGACTGTGGAT R: CCAAGCTGGTGGCAAGGAAG	94°C, 30 s; 60°C, 30 s; 72°C, 30 s; 36 cycles

PCR primers for potential off-target CDS for *P11*-gRNA2 in mice

Gene names	**Primer sequences (5'-3')**	**PCR conditions**
Lrp2	F: CCTACAGGGCCATAGGAGTA R: TCATGAGAAGGCTGAAGAGA	94°C, 30 s; 60°C, 30 s; 72°C, 30 s; 36 cycles
Orai3	F: GGCACCCATGGGTAAACCAG R: GATCCCTGGGGTTTCTGAGG	94°C, 30 s; 55°C, 30 s; 72°C, 30 s; 36 cycles
Nrg1	F: TTCAAAGGAGGAGTCGAGGA R: GCAGGCTAAAAGGAACGATG	94°C, 30 s; 60°C, 30 s; 72°C, 30 s; 36 cycles

PCR primers for identification of on-target indel mutations of *P11* in MEFs

Primer names	**Primer sequences (5'-3')**	**PCR conditions**
Crispr-MEF	F: TCCAATACCTGAAGTGTCTGT R: GGTGGAATCTTTTCCACTTTG	94°C, 30 s; 60°C, 30 s; 72°C, 30 s; 36 cycles

PCR primers for potential off-target CDS for *P11*-gRNA in MEFs

Gene names	**Primer sequences (5'-3')**	**PCR conditions**
Adar	F: CAGTGGGACCGAGGAAGGTA R: ATCAATGCCTCTGGCCTTTT	94°C, 30 s; 60°C, 30 s; 72°C, 30 s; 36 cycles
Ripk4	F: GGGCATGTCACAGCATATCC R: CCTGTGACACTGGAAAAGCC	94°C, 30 s; 60°C, 30 s; 72°C, 30 s; 36 cycles
Mprip	F: ATACTTAGCCAGTGTTGGGG R: CTGTTCCTCAGGTTCCAGTG	94°C, 30 s; 60°C, 30 s; 72°C, 30 s; 36 cycles

Table S2. Prediction of off-target sequences for *P11*-gRNAs

Target sequences (5'-3')	**PAMs**	**Predicted off-target sequences (5'-3') [a]**	**Genes**	**Loci**	**Off-target analysis [b]**
ATCCCAA	TGG	ATCGCAGTTGGAGCACGCCA	NM_001014981	chr18: +64095538	0/5
ATGGAGC		ATCCCAAACAGAGCAGGCCA	NM_028783	chr9: +37213470	0/5
ACGCCA		TTCCCCAAGGGAGCACACCA	NM_181820	chr7: +3628285	0/5
CCTGAGA	GGG	CTTGGAAGAGCTCATGGAAC	NM_001081088	chr2: +69293004	0/5
GTGCTCA		CCTTTGCGTGCTCACGGAAC	NM_178591	chr8: +32941816	0/5
TGGAAC		TGTGATAGTGCTCATGGACC	NM_198424	chr7: +134917525	0/5

ACCTGAG	CGG	TTCTGGGAGTGCTCATGGAG	NM_019655	chr3: -89555877	0/5
AGTGCTC		GCCTGATAGGGCTCATGCAA	NM_023663	chr16: -97969665	0/5
ATGGAA		ACCAGAGAGTATTCTTGGAA	NM_201245	chr11: +59509712	0/5

[a] The off-target sequences in red color indicate different bases from on-target sites.

[b] At least 5 recombinant clones were sequenced and no off-target effects were observed.